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COMPARISON OF COMPOSITES AND AVERAGES WITH RESPECT TO BAKING QUALITY

I. PURE SAMPLES OF ONE VARIETY[1]

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Abstract

Comparisons of the loaf volume of composite samples with the average loaf volume of the individuals comprising the composite were made on three groups of samples: namely, (1) samples of pure Marquis grown in one locality in one season, grouped on the basis of protein content; (2) samples of pure Marquis, Reward, and Garnet in separate series grouped on basis of protein, irrespective of locality of origin; and (3) samples of the above three varieties grouped on basis of origin, irrespective of protein content.

In these studies the composite samples were all made up after the individual flours had been baked and, therefore, there was admitted an error due to the time factor in respect to age of the flours and also in respect to variability in baking technique. Despite this, however, there was found a very close agreement between the values obtained with the composite samples and the average values of the component flours. There were a large number of cases in which there was practically complete agreement between the two values and only a few in which the differences were very great. The correlation of the two values was on the average of the order of +0.95 and the conclusion was reached that the data obtained with the composite samples could be used safely as an estimate of the average values and *vice versa*.

The question whether or not a composite sample of wheat or flour gives as accurate information regarding baking quality as could be obtained by taking the average values of a number of individual tests, is one of very considerable interest to cereal chemists. If it could be demonstrated that the quality of a composite made up of twenty different samples of No. 1 Northern Marquis wheat were equal to the mean value of the individual tests, an enormous amount of time and labor might be avoided in certain types of investigation. As an instance we might consider the comparison of five varieties of hard red spring wheat. The ordinary procedure is to obtain samples of these varieties grown in many different localities, mill and bake them individually and then

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take the average of the results so obtained as a measure of the relative value of the respective varieties. If we could be certain that mixing the individuals of one variety would result in no complementary action, and provided we were not interested in comparing degree of variability, it would be much simpler to mix all samples of each variety, making thus five composite samples.

The cereal chemist who judges the quality of a wheat crop in a given area by reference to the average of his tests of individuals, tacitly assumes that the average value is an estimate of the value of the composite because he knows that the separate lots tested will eventually be made into composites, represented by the cargo, to be sold and used as such.

On the other hand, cereal chemists often judge the average value of a crop by the use of composites. Each year in Western Canada the quality of the crop is estimated and the relative value of the grades determined by testing the so-called "average" samples collected at various inspection points. These "average" samples are in reality composites made up by combining small aliquots according to the grade. If all the carloads of wheat represented in the composite sample were thoroughly mixed together and cargo shipments were made from that one bulk, doubtlessly the milling and baking test would give a reliable estimate of the particular class of wheat. This, however, does not occur because the car lots represented in the "average" become assigned to various elevators and go into various combinations to make up different cargoes. In using the quality as determined on the inspection point "averages" to judge the whole crop, therefore, there must be made the assumption that the quality of the composite fairly represents the average quality of the number of separate parcels that may be made up from the whole class. To put the case more concretely; suppose an "average" No. 1 Northern sample from Winnipeg

TABLE I

A COMPARISON OF SOME COMPOSITES AND AVERAGES

50% Hard red spring wheat flour plus 50% of	Loaf vol. cc.	Average L.V. of individuals	Composite as % of average
Australian flour	635	622	102
English flour	562	560	100
Durum of 1927	600	572	105
Durum of 1928	590	510	116
Durum of 1929	645	585	110

is made up of portions of 1,000 cars of that grade. These 1,000 cars conceivably might be used in making up five separate composite cargoes of No. 1 Northern wheat. To judge these parcels by the quality of the original "average" sample would mean assuming that it represents the average of samples obtained from the respective cargoes. In other words, it is taken for granted that there is very little if any complementary action when various lots of the same class of wheat are mixed together.

Thus we have on one hand, estimates of composites being made from averages, and on the other, estimates of averages being made from composites.

If the same conclusions can be reached by either procedure, it obviously would be very advantageous in many instances to choose the use of composites on account of the saving of labor.

Aside from the loss of information regarding variability, the principal objection to composites has been the belief that in mixture the component flours tend to complement each other and give results essentially different from the averages of the individuals. It can be easily demonstrated that this takes place in certain types of mixture and not in others. A few cases are given in Table I.

With the Australian flour there is evidence of a slight difference between the composite and the average of the components. With the durums the difference is quite marked and it varies with the different crops. With the flour milled from native English wheat there is no apparent difference between the composite and the average values. The greatest difference is found, in the cases cited, when flours from those wheats having the most diverse characteristics are mixed. The problem is to ascertain where to draw the line between wheats that may and may not be mixed without this complementary effect occurring. Can we safely mix samples of one variety, or of one class, or wheats from a given area, or only wheats of a certain protein content? In order to find an answer to these questions we have started with the simplest sort of mixtures and from there tried mixtures that promised to be more and more incompatible, or more likely to give complementary effect. As the first phase of this investigation there has been made a comparison of composites and averages of samples of a single pure strain grown in a very limited area and grouped on the basis of protein content.

Pure Samples of One Variety Grown in One Locality

The material consisted of 98 samples of pure strain Marquis wheat grown in one locality. These were milled on an Allis-Chalmers experimental mill to a straight flour representing about 95% of the total. After aging in cotton sacks for two months they were baked by the basic and bromate formulas with certain modifications described by Larmour and MacLeod (2).

TABLE II

COMPARISON OF COMPOSITES AND AVERAGES OF PURE STRAIN MARQUIS SAMPLES GROWN IN ONE LOCALITY

N.	Protein class, %	Basic L.V., cc.			Bromate L.V., cc.		
		Composite	Average	Diff. C-A	Composite	Average	Diff. C-A
4	9.0 - 10.9	550	548	2	565	586	−21
12	11.0 - 11.9	538	522	16	590	603	−13
24	12.0 - 12.9	560	538	22	630	646	−16
16	13.0 - 13.9	560	559	1	673	706	−33
17	14.0 - 14.9	572	573	−1	775	782	− 7
22	15.0 - 15.9	582	574	8	770	789	−19
3	16.0 - 16.9	593	598	−5	825	841	−16

After baking, these samples were stored in tightly covered tin cans for several months. The composites were then made up by combining flours from wheats of similar protein range. They were classified according to wheat rather than flour protein because our other studies of the relation of protein and quality have been made on this basis, and it therefore proved to be the more convenient. The combined flours were very thoroughly mixed, sampled and then baked in triplicate. Table II gives the values obtained for the composite flours together with the average values calculated from the individual tests. The data are shown graphically in Fig. 1.

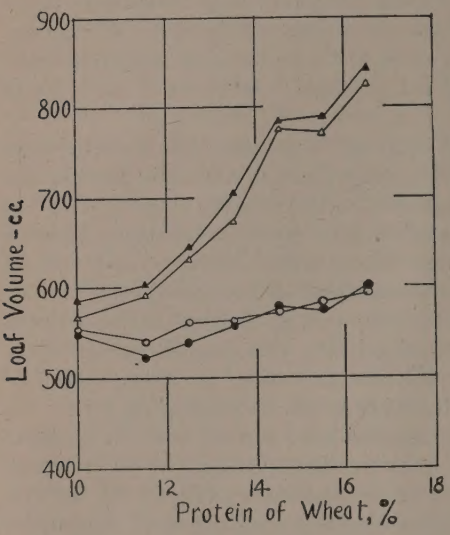

FIG. 1. *Comparison of composites and averages of pure strain Marquis samples grown in one locality.* ▲—*Average loaf volume by bromate formula.* △—*Composite loaf volume by bromate formula.* ●—*Average loaf volume by basic formula.* ○—*Composite loaf volume by basic formula.*

Comparison of the curves for the basic data indicate that the results are in very close agreement, the widest variation being 22 cc. found in the class, 12.0—12.9%. The mean difference is 6 cc. in favor of the composite.

With the bromate data there is a fairly consistent difference between the two sets of results, the composite being lower than the average by a mean difference of 18 cc. There is, however, a closer relationship between the bromate values than between the basic values. This is shown by the respective correlation coefficients.

		Basic	*Bromate*
$r_{a.c.}$	=	+0.9458	+ 0.9969

From Table V. A., Fisher (1), $P = 0.01$ when the value of $r = 0.8745$. These coefficients may therefore be considered significant.

With the bromate formula there appears to be a quite constant difference between loaf volume of the composites and the average loaf volumes. It can scarcely be attributed to change in baking technique as the results by the basic formula do not support such a supposition. It might represent a uniform decrease in strength due to age, or some sort of complementary effect resulting from mixing. No definite answer to this question is forthcoming at present.

From the results of this study it has been concluded that for comparing classes of one strain of wheat grown in a limited locality, the composite samples yield practically the same relative values as those obtained by calculating class averages from the tests of individuals. If any complementary effect occurs

it is slight and certainly not great enough to lead to any serious error in estimating the relative average value of the various classes.

Pure Strain Samples of One Variety Grown in Various Localities and Grouped on the Basis of Protein

Having shown that samples of one variety grown in one locality show good agreement between the loaf volume of the composites and the average loaf volume of the individuals, the next step was to examine this relationship for samples grown in widely differing localities. It is known that environment affects the quantity of protein but we do not know if in one variety there would be qualitative differences sufficiently great to produce complementary effect when the different flours are mixed together.

In this study there were used 138 samples of Marquis, 83 samples of Reward and 89 samples of Garnet, respectively, in three series. All samples were grown in Saskatchewan in the season of 1929. As in the previous study the grouping was based on protein of wheat using increments of 1%. The average loaf volumes and loaf volumes of the composites as well as the baking scores for the three series are given in Table III and shown graphically in Fig. 2, 3 and 4.

It can be seen that these data differ from those previously discussed in one important aspect, namely, that the values for the composites are not consistently lower than the averages. This indicates that the differences are

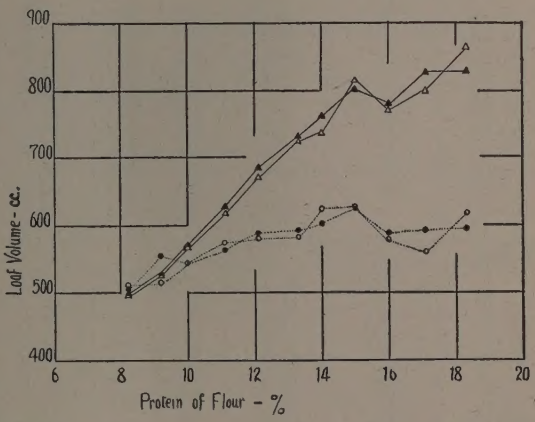

FIG. 2. *Comparison of composites and averages of Marquis samples grouped on basis of protein irrespective of locality.*—▲, △, ●, ○—*as in Fig. 1.*

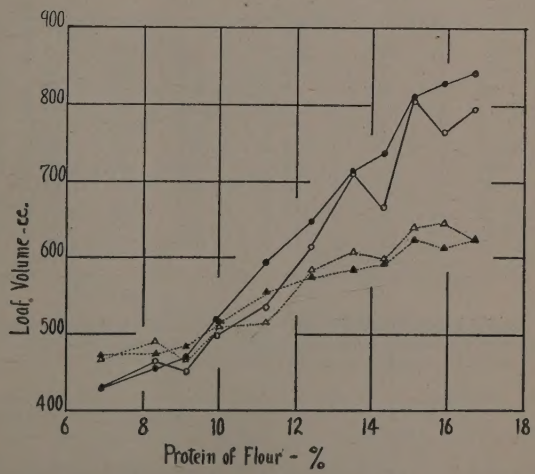

FIG. 3. *Comparison of composites and averages of Garnet samples grouped on basis of protein irrespective of locality.*—▲, △, ●, ○—*as in Fig. 1.*

TABLE III

COMPARISON OF AVERAGES WITH COMPOSITES GROUPED ON PROTEIN BASIS IRRESPECTIVE OF LOCALITY OF ORIGIN

Wheat protein class, %	No. in comp.	Protein of flour composite	L.V. Basic		C.B.S. Basic		L.V. Bromate		C.B.S. Bromate	
			Ave.	Comp.	Ave.	Comp.	Ave.	Comp.	Ave.	Comp.
Marquis										
8– 9	6	8.2	504	505	62	60	500	495	67	66
9–10	5	9.2	554	515	73	68	526	525	70	77
10–11	11	10.0	545	543	76	75	569	568	89	88
11–12	15	11.1	563	573	82	87	627	618	101	100
12–13	12	12.1	588	580	85	87	686	670	113	112
13–14	28	13.3	593	583	86	86	731	725	122	124
14–15	22	14.0	604	625	89	94	762	735	128	126
15–16	14	15.0	626	628	91	92	803	815	136	140
16–17	12	16.0	588	580	84	84	779	770	131	131
17–18	8	17.1	592	560	90	77	827	800	141	137
18–19	5	18.3	595	618	83	90	827	865	136	149
Reward										
9–10	3	8.9	532	540	68	66	498	495	60	55
10–11	4	9.9	540	530	76	70	550	548	82	76
11–12	6	11.0	558	548	82	75	611	603	95	94
12–13	7	12.1	586	598	88	86	694	665	115	109
13–14	10	13.1	608	588	91	86	717	735	121	123
14–15	11	14.4	609	620	85	90	798	793	135	134
15–16	19	15.4	647	673	96	102	837	880	142	151
16–17	13	16.3	665	700	99	109	883	930	152	160
17–18	7	17.4	674	728	105	117	937	913	159	158
18–19	3	17.9	645	655	94	96	933	945	154	164
Garnet										
7–8	5	6.9	470	468	47	50	428	428	37	39
8–9	7	8.3	472	490	50	55	455	465	45	44
9–10	8	9.1	484	468	58	54	470	448	56	46
10–11	12	9.9	516	510	63	66	519	498	68	65
11–12	7	11.2	554	515	78	68	594	535	92	82
12–13	12	12.4	574	583	78	88	648	613	104	100
13–14	13	13.5	585	608	80	87	713	710	117	122
14–15	10	14.3	596	598	85	83	737	665	123	114
15–16	7	15.1	625	640	91	94	813	805	136	141
16–17	6	15.9	614	645	92	96	830	765	140	133
17–18	2	16.7	625	625	94	94	842	795	141	134

TABLE IV

CORRELATION BETWEEN THE AVERAGE LOAF VOLUME AND LOAF VOLUME OF THE COMPOSITE

Variety	N.	r				1% point* of r
		Basic		Bromate		
Marquis	11	+0.888	±0.043	+0.989	±0.004	0.735
Reward	10	+0.975	±0.011	+0.989	±0.004	0.765
Garnet	11	+0.964	±0.014	+0.986	±0.006	0.735

*Table V.A., Fisher (1).

probably due to experimental error to a greater degree than in the other series. This may be accounted for by the fact that less time elapsed between the baking of the composites and the individuals.

In order to show more clearly the close relationship of the composite and average bromate values we have plotted the one against the other in Fig. 5. As the same units are used on both axes, a line of 45° slope would indicate equality of the two sets of values. This has been drawn in and the data actually show a close approximation to this curve. It is evident that prediction of the average based on the values obtained with composite samples would not be greatly in error for the whole range of protein studied.

The correlation coefficients are given in Table IV and they indicate very close relationship of the two sets of values. It seems justifiable, therefore, to conclude that with sound samples of a given pure variety of one season, the loaf volume of the composite samples may be regarded as an excellent estimate of the average values to be expected and *vice versa*.

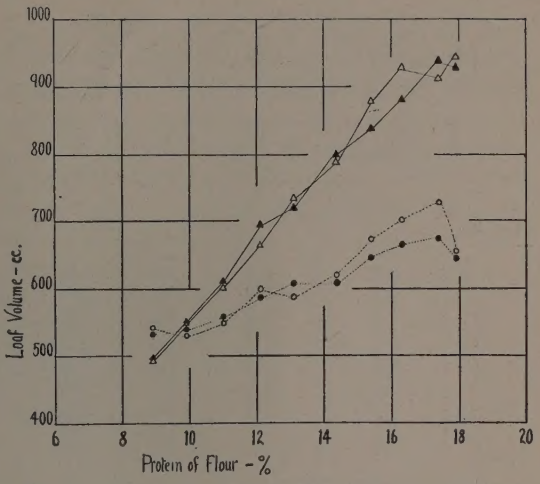

FIG. 4. *Comparison of composites and averages of Reward samples grouped on basis of protein irrespective of locality.* ▲, △, ●, ○–*as in Fig. 1.*

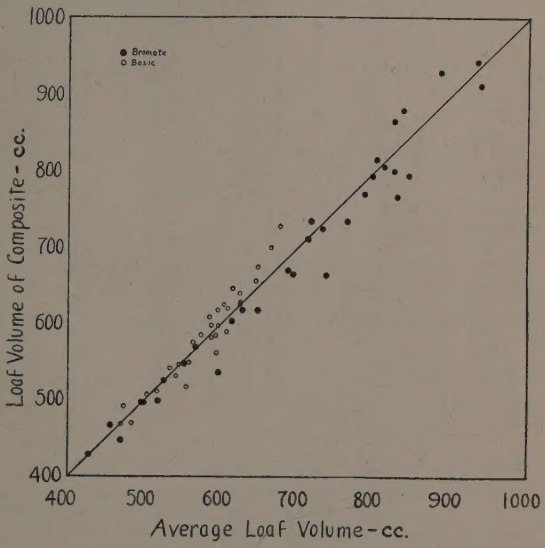

FIG. 5. *Comparison of composites and averages of Marquis, Reward, and Garnet samples grouped on basis of protein.*

Pure Strain Samples of One Variety Grouped on Basis of Locality of Origin

In both studies heretofore considered the samples were grouped on the basis of protein content of wheat. As it is generally considered that environmental

TABLE V

COMPARISON OF AVERAGES AND COMPOSITES GROUPED ON BASIS OF AREA OF ORIGIN

Area	No. in composite	Protein of flour composite	L.V. Basic			L.V. Bromate		
			Ave.	Comp.	Diff. A-C	Ave.	Comp.	Diff. A-C
Marquis								
I	8	11.8	537	535	2	604	593	11
II	14	10.2	545	548	−3	571	568	3
III	9	13.6	614	608	6	783	755	28
IV	25	14.2	599	593	6	741	710	31
V	9	16.2	603	602	1	785	795	−10
VI	16	15.8	598	613	−15	795	815	−20
VII	14	13.9	594	588	6	756	765	− 9
VIII	12	12.4	598	590	8	698	670	28
IX	10	13.2	583	590	− 7	709	730	−21
X	10	13.2	604	603	1	707	700	7
Reward								
I	12	13.1	599	635	−36	708	695	13
II	14	11.9	577	568	9	652	628	24
III	6	14.5	611	603	8	796	773	23
IV	11	15.5	648	670	−22	843	878	−35
VI	7	17.4	639	653	−14	869	868	1
VII	7	15.5	649	650	− 1	852	868	−16
VIII	11	13.9	614	623	− 9	770	768	2
IX	6	15.3	623	700	−77	815	853	−38
X	9	15.3	659	643	16	827	818	9
Garnet								
I	12	10.2	513	505	8	533	490	43
II	16	8.6	495	485	10	475	443	32
III	7	12.1	569	558	11	665	598	67
IV	9	13.5	597	573	24	718	645	73
VI	3	16.1	613	620	− 7	825	838	−13
VII	8	13.0	596	603	− 7	707	668	39
IX	4	13.2	575	615	−40	720	695	25
X	8	12.4	568	593	−25	660	638	22

TABLE VI

CORRELATION BETWEEN LOAF VOLUME OF THE COMPOSITE AND THE AVERAGE LOAF VOLUME FOR THE SERIES GROUPED ON BASIS OF ORIGIN

Variety	N.	r		1% point* of r
		Basic	Bromate	
Marquis	10	+0.961	+0.971	0.765
Reward	9	0.657	0.976	0.798
Garnet	8	0.913	0.978	0.834

*Table V. A., Fisher (1).

conditions profoundly affect the strength of wheat, one might suppose that samples having the same protein content might have been grown under nearly the same environmental conditions, and that therefore they would show similar characteristics. The observation that composites check the average values so closely, confirms this supposition. In order to obviate this possibility, a number of composites were made up on the basis of locality of origin. The samples at hand were divided into ten groups representing as many areas into which the province had been subdivided. Comparison of loaf volumes of these composites and the respective average values is made in Table V and shown graphically in Fig. 6.

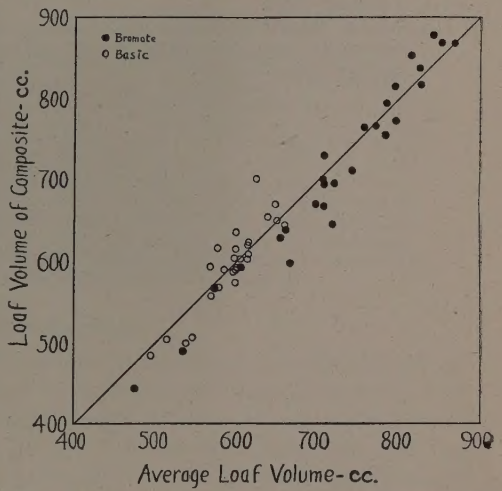

FIG. 6. *Comparison of composites and averages of Marquis, Reward, and Garnet samples grouped on basis of area of origin.*

The agreement between averages and composites is quite good for Marquis, by both the standard and bromate formulas. The Reward gives quite close agreement except in the case of Sample 9 which is 77 cc. greater than the average value for the basic baking. The Garnet basic results are not any too close but the agreement is good enough for the purpose of predicting one value from the other with a reasonable degree of accuracy. The Garnet bromate data show four cases of very poor agreement. It should be noted too that in all but one case the average value was greater than the value obtained with the composite. No reasonable explanation for this is apparent.

As can be seen from Fig. 6, the relationship between composites and average values is very close. The correlation coefficients for the three varieties are given in Table VI. The value +0.657 for the Reward basic data is not significant, due to the wide discrepancy in values for Sample 9, but all the others show a high degree of correlation, indicating that the two variables are very interdependent. It appears safe, therefore, to conclude that either the composite or average loaf volume may safely be used to predict the other with a reasonable degree of accuracy.

This means that in area surveys for a single season, where one is dealing with one variety of wheat, a very great amount of time and work can be avoided. In place of milling and baking hundreds of samples, and calculating the average values, composites consisting of aliquot portions can be prepared and tested to give information as adequate as obtainable from the average of the individual tests. The use of the composite sample recommends

itself furthermore inasmuch as, with the great reduction of samples to be handled, many more tests can be made and the net result will be a more complete and comprehensive estimate of the character of the wheat in hand.

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VARIETAL TRIALS, PHYSIOLOGIC SPECIALIZATION, AND BREEDING SPRING WHEATS FOR RESISTANCE TO *TILLETIA TRITICI* AND *T.LEVIS*[1]

By O. S. Aamodt[2]

Abstract

There has been a considerable increase in the amount of bunted wheat in western Canada recently. One hundred and forty-nine varieties and selections of spring wheat showed all gradations in reaction to this disease when inoculated artificially, varying from apparent immunity to high susceptibility. The increase in bunt can be accounted for in part by the use of certain varieties that are more susceptible than some of those grown formerly. There has also been an increase in the number and virulence of physiologic forms. One physiologic form of *T. tritici* and five of *T. levis* were obtained from six collections of bunt in this study. The isolation and study of relatively pure forms of the organism will be necessary for a study of the genetic factors in the host governing the reaction to bunt. Inheritance studies at present indicate that multiple factors, the exact nature of which has not yet been determined, govern the reaction to this disease. Production of resistant varieties suitable for the prairie provinces of Canada offers a very promising means for reducing the losses due to bunt of wheat.

Introduction

There is a great need in western Canada for improved varieties of hard red spring wheats that are resistant to such destructive diseases as rust, foot- and root-rots and smut. In the province of Alberta destructive epidemics of stem rust are very infrequent but the foot-rots and smuts are more destructive as well as more frequent in occurrence. In the wheat improvement program at the University of Alberta these latter diseases are being given primary consideration.

Bunt, or stinking smut, of spring wheat caused by *Tilletia tritici* (Bjeck.) Wint. and *T. levis* Kühn appears to be increasing rapidly in western Canada in recent years (22). This increase in prevalence may be attributed to several causes. First, the growing of varieties of wheat that are more susceptible to bunt than some of the older standard varieties such as Marquis. Second, an increase in the number, prevalence and distribution of more virulent strains of the bunt organism. Third, the failure of farmers to treat their seed regularly, or the practice of treating carelessly and inefficiently. Fourth, variation in quality of the fungicides and the consequent ineffectiveness of the treatments. Data concerning the first two of the above-mentioned causes of the increase in bunt are presented in this paper. A preliminary report on the results obtained in an attempt to produce resistant varieties by hybridization is also included.

In many sections of the wheat growing areas on this continent the heavy annual losses of wheat and the lowering of commercial grades by bunt point to the need for more certain means of bunt control. The possibility of seed injury under certain conditions, and the expense of seed treatment would be largely eliminated through the use of resistant varieties. The growing of

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Contribution from the Department of Field Crops, University of Alberta, Edmonton, Canada, with financial assistance from the National Research Council of Canada.

[2] *Professor of Genetics and Plant Breeding, University of Alberta.*

wheats immune from bunt would insure greater production without materially increasing the cost.

The first step in the production of resistant varieties is to test all available varieties with the hope of finding some that are naturally resistant to bunt. The second step is to select as parents for crossing such varieties and strains as are most likely to produce progeny some of which are both resistant to bunt and desirable commercially. Owing to the need for the development of varieties resistant to all diseases that are likely to be limiting factors in production, the second method is proceeded with directly on the basis of the knowledge available from other breeding studies. The synthetic production of new varieties through the use of composite crosses seems to be the logical method of attack upon this complicated problem. General knowledge of the genetics of wheat, and of the mode of inheritance of the characters being dealt with in particular, are greatly needed. Such information would enable one to plan more intelligently and definitely the method of attack and would insure greater success in the production of varieties with particular recombinations of characters.

Varietal Reaction

Extensive studies have been made on bunt resistance, especially of winter wheats. These studies have shown that there are wide differences in susceptibility between varieties. Woolman and Humphrey, 1924 (44), have summarized the early literature on varietal reaction to bunt. They report N. E. Tscharner as the first to note differences in varietal reaction to bunt as early as 1764. In 1901, William Farrer (13) reported the results obtained from inoculating ten varieties of wheat. Numerous observations and studies have been made since that time. More recently Vavilov, 1918 (43), Heald and Woolman, 1915 (25), Gaines, 1918-23 (17, 18, 19), Stephens and Woolman, 1922 (41), Johnston, 1924 (28), Coons, 1924 (9), Reed, 1924 (32), Faris, 1924 (11), Tisdale *et al.*, 1925 (42), Briggs, 1926 (4), Sampson, 1927 (39), Reichert, 1928 (36), and Heald and Gaines, 1930 (24), have made extensive studies on the reaction of wheat to bunt. These workers have dealt primarily with winter wheats.

Reports on the differential reaction of spring wheats, especially the hard red spring wheats, are comparatively meagre. Stakman, Lambert and Flor in 1924 (40), reported on an extensive study conducted over the five-year period 1919-1923. Their studies were on the reaction of spring wheats to *T. levis*. Rodenhiser (38) continued these studies and reported on the results obtained during the years 1924-1927. In 1929, Brentzel and Smith (2) reported on the varietal reaction of a number of spring wheats to both *T. tritici* and *T. levis*. In 1930, Holton (26) reported on the reaction of eight wheat varieties to *T. tritici*.

Stakman, Lambert and Flor (40) point out that bunt has been relatively unimportant in the hard red spring wheat area because of the resistance of the commonly grown varieties. Haynes' Bluestem and Preston wheats preceded Marquis and were "somewhat resistant." Marquis was fairly resistant

to their collection of *T. levis*, the highest per cent infection obtained being nine. Rodenhiser (38) found also that these varieties were fairly resistant. Kota was quite susceptible and it was pointed out that if Kota should be grown generally on account of its resistance to stem rust, the importance of the bunt problem would probably be increased. These investigators found that when working with *T. levis* it was evident that the durum wheats (*Triticum durum*) as a class were resistant.

In the spring wheat region of the United States a heavy epidemic of bunt occurred in 1924 resulting in an enormous loss of grain and lowering of the grade. Smut infested seed was evidently used by the growers in 1925 and 1926 with subsequent heavy losses. One of the unusual features of these epidemics was that durum wheats as a group were being attacked heavily. The results of Stakman, Lambert and Flor (40) as well as earlier observations in the field had indicated that durum wheats as a group were resistant. Samples of smutty wheat from the grain terminals at Minneapolis and Duluth were examined for their spore contents and it was found that almost all shipments of smutty, amber, durum wheat contained *T. tritici*, while only a few contained *T. levis*. The reverse was true of the hard red spring wheats where most of the smutty shipments of wheat contained *T. levis* and only a few contained *T. tritici*.

Güssow and Conners (21), in 1927, reported the results of a survey made from 27 samples of smutty wheat representing all the grain growing sections of the prairie provinces of Canada. They found that ten of the 27 samples contained mostly *T. levis* spores and originated south of a line running east and west between Winnipeg and Calgary. The other 17 samples contained mainly *T. tritici* and were found north of this line. Results of a similar nature, although not as marked, were reported by Hanna and Popp in 1930 (22).

Reichert (36), in Palestine, found that the durum wheats were not more resistant to bunt than the common wheats as was commonly held. They were actually more susceptible than the common wheats to the forms of *T. tritici* present in Palestine.

In a recent publication from North Dakota, Brentzel and Smith (2) demonstrate clearly the relationship between the two species of bunt on wheat and the commonly grown varieties of durum and bread wheats. The durums as a group were fairly resistant to *T. levis* as reported by Stakman, Lambert and Flor but were very susceptible to *T. tritici*. The reverse was true of most of the commonly grown hard red spring wheats. Kota, Ceres and Progress were highly susceptible to both species of bunt.

Holton (26), in 1930, demonstrated also that the increase in severity of bunt on durum wheats could be explained by the presence of unusually virulent strains of *T. tritici*.

Owing to the severity of black stem rust epidemics in Manitoba there has been a great increase in the percentage of the wheat acreage devoted to the growing of durum wheats, these wheats, in general, being less severely injured by stem rust. In 1928, 16.5% of the durum wheat graded smutty (22). Here

again the durum wheats were found to be infested almost entirely with *T. tritici*. This instance provides a clear demonstration of how a new variety may greatly aggravate the losses due to a particular disease because of its greater susceptibility to the causal organism.

Several varieties of common wheat have been introduced also into the hard red spring wheat area of Canada and the United States in an attempt to reduce the losses due to black stem rust. These are Kota, Ceres and Progress. They are all somewhat resistant to stem rust but in addition to being susceptible to *T. levis* like the older varieties of common wheat they are also highly susceptible to *T. tritici*. These new common wheat varieties then, together with the durum wheats, have greatly aggravated the bunt problem, not only because of the inability of the varieties to withstand attacks by the bunt organism, but because they have been a medium through which the pathogene has become more thoroughly and widely distributed.

As a preliminary to the improvement of the hard red spring wheats at the University of Alberta all of the common varieties of spring wheat were tested for their reaction to various collections of bunt found in the province. The work was started in the spring of 1929, but owing to the drought, the infection percentage was too low and variable to be reliable. In the spring of 1930 conditions were more favorable for bunt infection, as is indicated by the high average infection percentage obtained on susceptible varieties.

In an earlier publication on breeding wheat for stem rust resistance (1) the writer has pointed out that, "crop improvement which is concerned with disease resistance must take into consideration: (a) the possible existence of physiologic forms of the pathogenic organism, (b) the need of a survey to determine the prevalence and distribution of the various forms, (c) the varietal reactions of the host to particular forms, (d) the reactions between host and parasite as definite genetic characters, and (e) the possibility of combining the resistance to all forms within a single desirable commercial variety." Investigations on the bunt problem have not advanced sufficiently to isolate the various natural divisions and organize a coherent plan of attack. In order to assure that any new wheat productions might be reasonably resistant to the forms prevalent in the province, the practice of using composite chlamydospore cultures as inoculum was employed. Inoculum was obtained from four general sources as follows: (a) infected heads of a number of varieties in the experimental plots at the University of Alberta, Edmonton, (b) material collected in the fields at various places in the province, (c) samples of smutty wheat from the Dominion Grain Inspection Office, Edmonton, and (d) samples of infected heads sent in by growers and other agencies.

It is recognized that the use of composite cultures of inoculum brings in a possible deviation in the infection percentages from that which might have been obtained if pure cultures were used. Heald (19) has demonstrated that spore load is an important factor in obtaining the maximum percentage of infection. A composite culture may be expected to possess varying inherent potentialities for attacking different varieties of wheat. It is obvious that it

would be practically impossible to have all the parasitic entities which go to make up the composite culture in the same proportions, especially when the virulence of the individual pathogenes and the reactions of the separate hosts are unknown. Even though the numbers of spores of the different cultures are approximately equal in a composite culture, there is still the likelihood of differences in viability of spores of the different cultures. Consequently it is to be expected that if there is a differential reaction of the varieties to the pathogenic forms in the composite inoculum, the test might not be comparable to the infection obtained by the use of individual cultures or forms. In using a composite culture it would not be difficult to conceive of a mixture of forms containing potentialities for infecting a particular variety in entirely different proportions from that of a second variety. In order to overcome the possibility of not obtaining the maximum infection, cultures, or forms, of bunt are carried individually through several generations on the varieties of wheat commonly grown in the prairie provinces of Canada. These cultures are then used as inoculum in separate tests on the standard varieties, parental material and the more promising hybrid selections.

In general, the results reported in this paper would seem to indicate that the use of composite cultures of chlamydospores for testing varieties of wheat for their reaction to bunt are sufficiently reliable for all practical purposes. The infection percentages obtained on different standard varieties when composite cultures were used as inoculum are in fairly close agreement with those obtained by other workers (19, 20, 21) and from field observations. In one of the physiologic-form experiments, evidence was obtained to the effect that the sum of the percentages of infection obtained through the use of separate cultures was approximately equal to the percentage obtained from a composite culture made from the same material. Hope wheat when inoculated with six separate cultures at 10° C. had infection percentages as follows: No. 1—7; No. 2—13; No. 3—0; No. 4—0; No. 5—0 and No. 6—0. When this wheat was inoculated with a composite culture of these forms the infection percentage was 22, or approximately the same as the sum of the percentages from the separate inoculations. Naturally such a relationship could hold only for varieties that are resistant since the sum of infection percentages of susceptible varieties would be well over 100.

The seed of 137 varieties and hybrid selections was heavily smutted with a composite collection of both *T. tritici* and *T. levis* before sowing. Approximately equal amounts of inoculum were applied to the seed of each variety, or hybrid, in order to make the spore load as uniform as possible. The dry chlamydospores and the seed were thoroughly shaken in the envelope until the surface of each kernel had a dark smutty appearance. The inoculated seed was sown in the field in rows one foot apart. The furrows were opened with a garden cultivator and the seed dropped and covered immediately in order to avoid drying-out of the soil.

The percentages of bunt were determined by making counts of the smutted heads and the total number of heads from which the infection percentage was

calculated. The plots were not replicated in the field in this preliminary experiment, but replicate plantings were made in the greenhouse, some of which were kept under controlled temperatures for the first two weeks after sowing.

In order to obtain some indication of the need for replication in bunt tests, a series of sixteen Marquis rows were sown as checks both in the field and greenhouse experiments. In the greenhouse the seed was sown in small wooden flats. The soil temperature in the greenhouse was too high when the seed was germinating, and consequently the infection percentage was low. The greenhouse series of Marquis checks varied from 5 to 29% with an average of 17.5, and the field series from 38 to 88% with an average of 61.2. The data are presented graphically in Fig. 1.

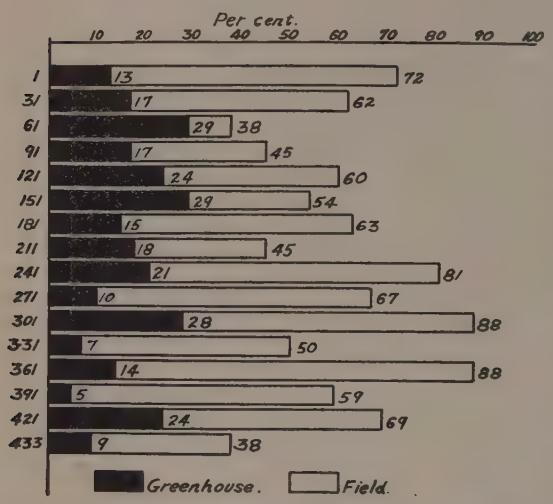

FIG. 1. *Bunt percentages in Marquis check plots grown in the greenhouse and in the field.*

These results demonstrate rather strikingly the need for replication in conducting experiments with bunt. The variations are greater than was expected in view of the fact that the Marquis seed was elite stock, and that the samples of both seed and inoculum were handled in an identical manner. The seed sown in the greenhouse and that sown in the field came from the same prepared sample. The correlation coefficient between field and greenhouse infections is $+0.1321 \pm 0.139$. This non-significant value is to be expected in view of the great degree of variability and the small number of tests.

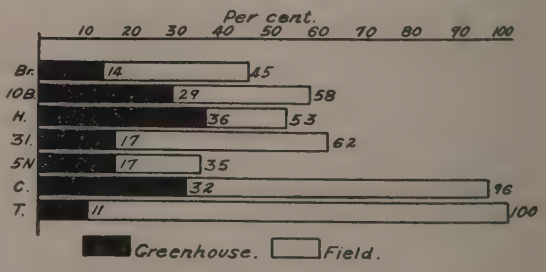

FIG. 2. *Bunt percentages on different strains of Marquis wheat in the greenhouse and in the field.*

Different stocks of an agronomic variety are usually considered as being practically equal in resistance to specific diseases. The soundness of this opinion is dependent to a considerable extent on the method of origin of the particular stock in question. Seed stock developed under the rigid rules and

regulations laid down by the Canadian Seed Growers' Association for the production of elite and registered seed might be expected to be uniform for most characteristics. Percentages of bunt infection were determined for seven strains of registered Marquis. The data are presented graphically in Fig. 2.

The results are similar to those obtained in the replicated plots from a single stock of Marquis. There is a range in infection from 11 to 36% in the greenhouse with an average of 22.3%, and 35 to 100% in the field with an average of 64.1%. One would not be justified in concluding that there is an inherent difference in the susceptibility of these strains of Marquis to bunt. The variability between individual plots is great. Average values from replicated plots of each strain are necessary before the inherent reactions to bunt of each strain could be determined.

The average infection percentage of the 149 varieties and selections was 15.3 in the greenhouse and 55.8 in the field. These figures approximate those of the Marquis checks in the same experiment. With the great increase in number of tests there is a significant correlation coefficient between the bunt percentages in the two tests of $+0.4602 \pm 0.044$. The correlation surface showing the distribution of the bunt percentages for the 149 plots of wheat varieties and selections grown in the field and the greenhouse is illustrated in Table I.

TABLE I

CORRELATION SURFACE SHOWING THE DISTRIBUTION OF THE BUNT PERCENTAGES FOR 149 WHEAT VARIETIES AND SELECTIONS GROWN IN THE FIELD AND IN THE GREENHOUSE

Percentages of field infection	Percentages of greenhouse infection: 0	5	15	25	35	45	55	65	Freq.
0	2								2
5	2								2
15	4	4	3	2					13
25		1		2					3
35	2	7	3	1	1				14
45	4	7	10	2					23
55	1	10	10	9	1				31
65	2	8	4	5	2				21
75	3	1	6	5	3				18
85	1		3	5	2	2	1		14
95		1	3		1	2		1	8
Freq.	21	39	42	31	10	4	1	1	149

$r_{xy} = +0.4602 \pm 0.044$

The correlation is high when one considers the great variation that was shown by the replicated check plots. The relationship between the results of the tests in the greenhouse and in the field indicates that these results are sufficiently reliable to be used as a preliminary indication of the reaction of the different varieties and selections to bunt. The infection percentages from the field tests of the commonly grown spring bread wheat varieties are given in Table II.

TABLE II

COMMON VARIETIES OF SPRING WHEAT SHOWING PERCENTAGE OF HEADS INFECTED WITH BUNT IN FIELD TESTS IN 1930

Variety	N.S.N.*	Percentages of bunted heads	Variety	N.S.N.*	Percentages of bunted heads
Red Bobs 222	I–0–18	92	Marquis	I–0–9	53†
Dicklow	I–30–4	88	Hard Federation	I–28–35	50
Reward	I–0–21	88	Reliance	I–29–4	49
Hard Federation	I–30–2	86	Renfrew	I–0–20	46
Kota	I–29–6	83	Huron	I–0–4	44
Red Fife	I–0–19	78	Marquis × Emmer (H_{35})	I–28–111	44
Progress	I–29–7	77			
Ceres	I–25–1	71	Marquis × Emmer (H_{44})	I–28–112	36
Preston	I–30–3	70			
Marquillo	I–29–8	65	Renfrew	I–0–20	33
Early Triumph	I–0–2	62	Kitchener	I–0–5	26
Producer	I–25–7	58	Ruby	S–22–42	18
Supreme	I–0–23	55	Garnet	I–0–3	12

*N.S.N. = Nursery stock number. †Av. 16 plots.

It will be noticed from a study of the data presented in Table II that the more recent productions, such as Kota, Ceres, Reward, Progress and Red Bobs 222, are all highly susceptible. Their percentages of bunt are 83, 71, 88, 77 and 92 respectively. The average of sixteen Marquis checks is 53%. These results indicate that the introduction and culture of new varieties less able to resist infection by the bunt organism, has contributed materially to the present increase in bunt on the farms in western Canada. Even though a new variety, such as Kota, meets with little favor from the grower, and is soon discarded, it is a most effective agent in disseminating the organism to farms and in regions where the disease had not previously been a particularly important problem.

The loose smut of wheat (*Ustilago tritici*) has received considerable attention during recent years. Its prominence can also be readily accounted for by the introduction of new susceptible varieties, such as Kota, Ceres and Reward. The seed of these new varieties distributed in western Canada was fairly heavily contaminated with loose smut. In preliminary variety tests Kota and Ceres have shown themselves to be highly susceptible to loose smut. Reward has often been referred to as a very susceptible variety. This can be accounted for by the fact that a portion of the first lots of seed that were distributed were contaminated. In varietal tests conducted by the writer where the wheats have been artificially inoculated, Reward has shown itself to be not nearly as susceptible as Kota and Ceres, and only slightly more so than Marquis.

Reward wheat is a very desirable type of hard red spring wheat for certain portions of western Canada because of its earliness and excellent milling and baking qualities (30). While considerable objection has been raised to it because of its susceptibility to loose smut, what is probably more important as a problem in the near future is its susceptibility to bunt. Fortunately the seed originally distributed seems to have been free from this disease. Bunt is

an accumulative disease. It may take several years before Reward is sufficiently contaminated to be called to the attention of most growers. Contact with bunt spores through machinery, bins, sacks and mechanical mixtures with other susceptible varieties on the farms will undoubtedly bring about in time a contamination of Reward. Its high degree of susceptibility as demonstrated by the tests reported in this paper and as indicated by a number of samples already received from growers, points to the necessity of regular seed treatment of this variety whether the stock is already contaminated or not.

Several of the newer varieties have brought into prominence another important factor in the spread of bunt, to which some reference has already been made. The results of a number of the investigators working on spring wheats (2, 26, 40) indicate that the durums, as a class, are less susceptible to *T. levis* and more susceptible to *T. tritici* than the hard red spring wheats. The reverse is true of the hard red spring wheats; their varieties have been less susceptible to *T. tritici* and more susceptible to *T. levis* than the durum wheats. Ceres, Progress and Kota are highly susceptible to both species of bunt and consequently may be important in the distribution of both of them. There is a great need for more exact and detailed knowledge regarding the reaction of new varieties previous to their distribution to the grower.

Several of the hybrid selections tested were free from infection in both the field and the greenhouse trials. These are to be tested further and if they continue to remain free from infection or are highly resistant they will be used as parental material in crosses.

Garnet wheat, while not immune, was the most resistant of the varieties commonly grown in western Canada. It had an infection of only 12%. These experimental results conform with observations in the field where it has been noticed that Garnet seldom contains bunt. Garnet was observed to be highly resistant to five of the six collections of bunt with which it was inoculated in the physiologic-form experiments (Fig. 9), and in the breeding studies where thirty replicated parental check rows had an average of 22% bunt when a composite inoculum was used (Table V). Physiologic form 2, a strain of *T. levis*, was much more virulent on Garnet than any of the six collections used in the physiologic-form experiment. Form 2 produced 56% bunt on Garnet. Physiologic form 1, a strain of *T. tritici*, produced only 7% under similar conditions.

One case of a naturally infested field of Garnet with 7% of the heads infected was observed at Egremont, Alberta, in 1930. The organism in this case was *T. tritici*. The echinulations on the spores were much more pronounced than on other collections of *T. tritici* made in the same year. A preliminary test was made with this collection in the laboratory and greenhouse, and Garnet was infected to the extent of 82 and 87% respectively. This collection is evidently much more virulent than form 1 on Garnet, and also on some of the other differential hosts. In the laboratory tests Hope had 71% of the plants infected and Mindum (a durum wheat), 86%.

Physiologic Forms

The discovery of physiologic forms of phytopathogenic organisms has contributed a wealth of knowledge to the understanding and control of plant disease by the production of resistant varieties. Knowledge concerning the number, prevalence and distribution of physiologic forms of any pathogenic organism is of such vital importance to the crop improver that he cannot afford to ignore or fail to give cognizance of the possibilities. To produce new varieties that are not resistant to all the forms of the pathogene which are present in the region in which the variety is to be grown is to acquiesce, at the best, to only temporary or partial success. The pathogenic forms present in those regions from which contamination is likely to arise, through such agencies as wind, water, etc., and the dissemination of the organism on the seed of the host, must also be considered. Plant quarantines are ineffective or only temporary in preventing the introduction of new inoculum where there is a natural exchange of materials, or where agencies which act as carriers in distributing the organism occur.

The existence of physiologic forms in the organisms causing powdery mildews and rusts, and in other plant pathogenes has been known for some time. Kniep (29), in 1919, was the first to show that there are physiologic forms in the smut fungi. The existence of physiologic forms in the smuts of cereals was first demonstrated in *U. hordei* (covered smut of barley) by Faris (12) in 1924, and by Reed (33) in *U. avenae* and *U. levis* (loose and covered smuts of oats). When studying the influence of various factors on the infection of wheat by *Tilletia tritici* and *T. levis* Faris (11) obtained some evidence of the existence of specialized races. His experiments were extended and the results were confirmed in 1927 (34) and 1928 (35). Miss Sampson (39) and Rodenhiser and Stakman (37) in 1927, have also presented some evidence of physiologic specialization in *Tilletia*. The latter working with the spring wheats Einkorn, Marquis and Kota as differential hosts compared the virulence of several collections of bunt from European countries with that of Minnesota collections.

In 1927, Stephens (20) at Moro, Oregon, showed that several wheats which had been practically smut free for several years, when inoculated artificially with local collections of bunt became heavily infected. Gaines (20) about the same time obtained a smut collection from Germany which was also very virulent on several of the same varieties infected by Stephens with his local bunt. These more virulent forms were shown to be strains of *T. levis* which species had not previously been prevalent in the state of Washington.

Roemer (20) working in Germany reciprocated with Gaines in the exchange of bunt collections and wheats. He likewise demonstrated the existence of several physiologic forms and suggested that each collection is probably a mixture of several pure lines.

Rodenhiser (38) in 1928 demonstrated again that several European collections of bunt and one from Egypt consisted of a number of physiologic forms of *T. levis*. He demonstrated also that several collections of *T. tritici* from

European countries, and one from New Zealand consisted of several physiologic forms. No evidence was presented regarding the existence of pathogenic forms from collections in the spring wheat area of the United States and Canada. Marquis, Kota, Mindum, Pentad and Einkorn, all spring wheats, were used as differential hosts to identify the physiologic forms.

Reichert (36) demonstrated that, in Palestine, forms of bunt were present which were especially virulent on the durum wheats.

Brentzel and Smith (2) in 1929, used nine varieties of durum wheat and twelve varieties of common wheat as differential hosts and found that the presence of virulent physiologic forms of *T. tritici* were responsible for the increase in prevalence of bunt on durum wheats. Common wheats, in general, were more susceptible to *T. levis* and the durum wheats more susceptible to *T. tritici*. The standard varieties of common wheat used as differential hosts were Hope, Preston, Marquillo, Reliance, Marquis, Ruby, Powers' Fife, Webster, Ceres, Progress, Haynes' Bluestem, Kota and Quality.

Holton (26) thought that in view of the increasing severity of bunt in the United States and the evidence contributed by Reichert, the presence of new and virulent strains of bunt was responsible for the outbreak of bunt on durum wheats in the spring wheat region. He used eight varieties of spring wheat as differential hosts, namely, Kota, Preston, Marquis, Marquillo, Hope, Mindum, Pentad and Vernal emmer. Three physiologic forms of bunt were found in the spring wheat region. One from Manitoba was especially virulent on Kota and of low virulence on the other seven varieties. A collection from North Dakota was especially virulent on the durum wheats, Mindum and Pentad. A third collection from Minnesota was distinguished by its higher relative virulence on emmer.

Heald and Gaines (24) have reported recently the results obtained from inoculating 22 wheats with seven separate collections of bunt. Their tests included several spring wheats among which were Marquis and Hope. Hope was immune from bunt and Marquis highly resistant when spring sown. Hope and Marquis were highly susceptible, however, when fall sown, indicating that climatic conditions and cultural practices are responsible for wide fluctuations in the degree of bunt infection. The trend in the states of Washington and Oregon over a period of years has been in the direction of increasing amounts of smut. This fact, combined with experimental evidence demonstrating that wheats which formerly were resistant are now susceptible, leads to the conclusion that new strains of bunt are responsible for some of the increase. Heald and Gaines call attention to the need for effective seed treatment to inhibit the spread and further development of these new and virulent forms.

Bressman (3) studied the behavior of 100 collections of bunt on 10 differential hosts and found six physiologic forms of *Tilletia levis* and four of *T. tritici*. Practically all of the varieties of wheat which had been classified formerly as resistant to bunt were susceptible to one or more of the physiologic forms. Hosar, a hybrid selection, was consistently resistant to all of the available collections of bunt fungi.

Holton (27) has recently confirmed his earlier work on the susceptibility of durum wheats to new forms of *T. tritici*. Evidence is also presented which shows that Vernal emmer and Marquis were more heavily bunted in 1930 than in 1929. It is concluded from these results that new and more virulent forms attacked these two varieties in 1930.

Most of the studies to date on the identification of physiologic forms of the bunt fungi have been made primarily with winter wheat varieties as differential hosts. The majority of these varieties are not sufficiently winter hardy to survive in the field in most years with the climatic conditions prevailing at Edmonton. When grown in the field from spring sowings, or in the greenhouse, the dormancy period of the winter wheats prolongs the experiment, or necessitates special treatment of the plants to avoid long dormancy periods. Consequently it would be very impracticable to use the usual winter wheat differential hosts for the identification of physiologic forms of bunt fungi in Alberta. With the rapidly increasing prevalence of bunt in spring wheats a number of investigators have selected spring wheats as differential hosts. Some of the varieties used by Rodenhiser and Stakman, Brentzel and Smith, and Holton were supplemented with a few others to use as differentials in these studies. The varieties are as follows: *T. vulgare*—Kota, Red Bobs, Bozosio, Progress, Preston, Marquis, Reliance, Garnet, Hope; *T. durum*—Pentad; *T. dicoccum*—White Spring Emmer; and *T. compactum*—Little Club.

The term "physiologic form" is applied in this paper to a purified chlamydospore collection of bunt capable of producing a definite set of reactions on a given group of wheat varieties. Flor (16) has demonstrated that *T. tritici* is heterothallic. Reduction division and segregation evidently take place before the fusion of sporidia to form the next sexual generation. Hybridization between monosporidial lines is necessary to bring about successful infection with subsequent chlamydospore production in the host. The sporidia which fuse and later produce chlamydospores may be similar except in sex. They may or may not have different parasitic capabilities, and for this reason the application of the term "physiologic form"—a term which implies a certain degree of homogeneity—to collections of chlamydospores might be questioned. It is to be expected that most collections would be mixtures of physiologic forms and capable of infecting a wide range of hosts. Few varieties make good differential hosts. Wheat varieties with varying capabilities for infection would act as screens to a certain extent in reducing the number of forms in a mixed collection. Dillon-Weston (10) has demonstrated that new forms may be isolated from the bunted wheat in England by using resistant hosts. The more resistant the host upon which a collection of chlamydospores is cultured the finer would be the screen through which the pathogenes would have to pass. A high degree of susceptibility would be more favorable to the carrying of a number of physiologic forms. Varieties more resistant, or selective, to parasitic invasion by particular forms would be better hosts upon which to maintain pure cultures. Using the same host for several successive generations would also be of considerable value. Briggs (6) has found a high degree of

stability and uniformity of reaction on particular hosts over a period of years and through numerous generations.

Considerable differences in virulence in chlamydospore collections have been demonstrated by a number of investigators. With consistently uniform reactions in a succession of generations, from most of the cultures it has seemed justifiable for all practical purposes to consider the different collections of chlamydospores varying in virulence as separate genetic entities. It is convenient and useful, therefore, to designate such collections of chlamydospores as physiologic forms.

Six collections of bunt were made on pure varieties of spring wheat in the experimental plots at the University of Alberta. The bunt on Red Bobs was *T. tritici* with a small contamination of *T. levis*, and the bunt on the other five varieties was *T. levis*. The seed of 12 varieties of wheat was inoculated separately with chlamydospores from each of the six collections of bunt. In addition one set of seed was inoculated with a composite culture made up of all the collections of bunt. A sixth set of seed of the 12 varieties was sown uninoculated as a check. Precautions were taken to avoid mixing of the inoculum in the preparation of the samples and in the sowing. Hands and equipment were thoroughly washed and disinfected between handlings of the different lots of material.

Faris (11) and Reed (35) report that low temperatures approximating 10° C. were the most favorable for obtaining high infection on susceptible varieties. As a preliminary to these studies four series of wheat were inoculated with a composite culture of bunt in order to determine the condition that would be most favorable for infection. Two series were sown in wooden flats filled with soil and then placed in freezing chambers and the seed germinated at controlled temperatures, one series at 5° C. and another at 10° C. The third series was sown in the field on April 23, when the soil temperature at seeding depth was approximately 10° C. The fourth series was sown in the field on May 15, when the soil temperature was approximately 15° C.

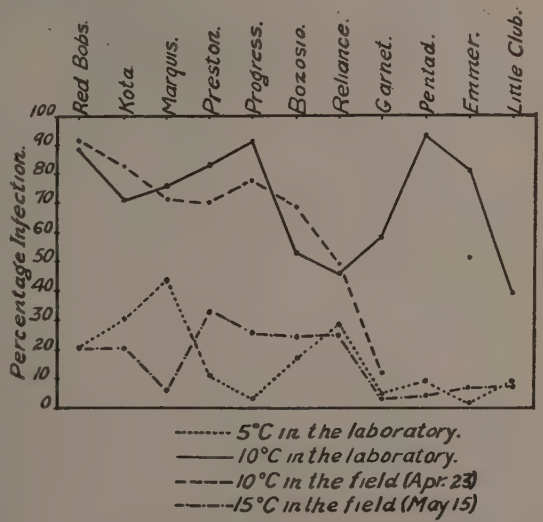

FIG. 3. *Bunt percentages on eleven varieties of wheat when the inoculated seed was germinated at different temperatures in the laboratory and field.*

Eleven varieties of wheat were used in each series except the early sown field series, which lacked three of the varieties. The average per cent infection for the eight varieties of wheat common to all four series was 65.4 when sown in the field on April 23 with a soil temperature of approximately 10° C. and only 19.6% when sown on May 15 with a soil temperature of approximately 15° C. In the laboratory the series germinated at 10° C. had an average per cent infection of 70.8 and only 20.0% when germinated at 5° C. The results obtained in this experiment are shown graphically in Fig. 3.

These results demonstrate and emphasize the importance of germinating seed inoculated with bunt spores at favorable temperatures for infection. A temperature of approximately 10° C. appears to be the most favorable for obtaining high infection on susceptible varieties. In these experiments the soil moisture was approximately 20-25%.

Six sets of the 12 differential hosts were inoculated with bunt collections from 1—Red Bobs, 2—Kota × Marquis, 3—Reliance, 4—Bozosio, 5—Ruskier, and 6—Saaminkil. The seed was sown in the field 1½ in. deep when the soil temperature was approximately 15° C. A seventh set of differential hosts remained uninoculated to serve as checks. The sets inoculated with the collections from Red Bobs, Kota × Marquis and Reliance were triplicated by sowing two additional sets in the laboratory and germinating the seed at 5° and 10° C. Uninoculated seed of each variety was sown again to serve as checks. Without exception the checks in all the experiments remained free from infection. The results are summarized in Table III.

TABLE III

PERCENTAGE OF BUNTED PLANTS IN 12 VARIETIES OF WHEAT INOCULATED ARTIFICIALLY WITH SIX COLLECTIONS OF *Tilletia* AND GERMINATED AT 10° AND 15° C.

Variety or differential host	Source of inoculum and percentage of bunted plants											
	1 Red Bobs		2 Kota × Marquis		3 Reliance		4 Bozosio	5 Ruskier	6 Saaminkil	Average at	
	10°C.	15°C.	10°C.	15°C.	10°C.	15°C.	15°C.	15°C.	15°C.	10°C.	15°C.
Kota	67	14	90	32	90	5	19	31	50	82	25
Progress	81	0	86	43	73	0	6	0	22	80	12
Bozosio	76	11	68	3	51	4	19	24	47	65	18
Red Bobs	60	41	63	61	41	0	8	10	46	55	28
Preston	56	15	35	28	59	0	0	50	31	50	21
Reliance	52	8	67	3	32	0	7	22	9	50	8
Marquis	65	0	23	12	40	4	29	40	5	43	15
Garnet	7	1	56	3	22	0	0	6	2	28	2
Hope	7	0	13	0	0	1	0	0	0	7	0
Little Club	38	15	62	0	32	0	67	0	0	44	14
Pentad	69	0	54	15	64	11	16	48	20	62	18
Emmer	50	0	69	20	70	5	7	12	13	63	10
Average	52.3	8.8	57.2	18.3	47.8	2.5	14.8	20.2	20.4	52.4	14.2

The infection percentages for collections 1 to 3 in Table III were taken from the series germinated in the laboratory at 10° C. The higher percentages of infection obtained in that experiment make it easier to differentiate between the reactions of the different collections and also make the comparisons more reliable. Collections 4 to 6, Table III, were studied in the field only where the soil temperature was approximately 15° C. at the time of seeding. The percentages of infection are not as high as were obtained with collections 1 to 3 but their reactions are markedly different on the various hosts.

The six collections of bunt showed approximately the same reactions on Kota, Red Bobs and Bozosio (Fig. 4-6). On the balance of the differential hosts shown in Fig. 7 to 12, the reactions of the six collections are sufficiently contrasted to be able to classify the collections as separate entities, or physiologic forms. In order to facilitate a detailed examination of the data the percentages of infection are classified as follows and summarized in Table IV.

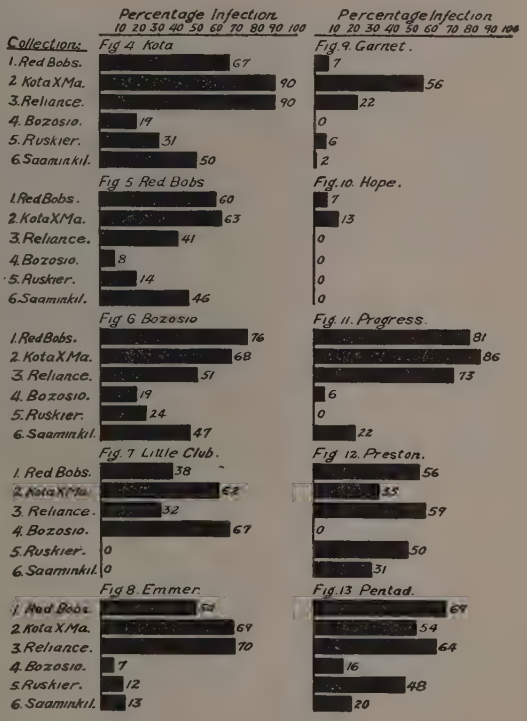

FIG. 4–13. *Bunt percentages on ten varieties of wheat when inoculated separately with six collections of bunt.*

0– 6% infection = highly resistant = HR
7– 15% infection = resistant = R
16– 30% infection = moderately resistant = MR
31– 45% infection = moderately susceptible = MS
46– 60% infection = susceptible = S
61–100% infection = highly susceptible = HS

The higher the degree of susceptibility the greater is the fluctuation in the percentages obtained, consequently it seemed reasonable to require greater differences the higher the susceptibility. The difference in infection percentage is 7 between the first two classes, 15 between the next three and 40 between the last two classes.

TABLE IV

THE REACTION OF TWELVE WHEAT VARIETIES INOCULATED WITH SIX COLLECTIONS OF *Tilletia*

Variety	Source of inoculum					
	1 Red Bobs	2 Kota × Marquis	3 Reliance	4 Bozosio	5 Ruskier	6 Saaminkil
Kota	HS	HS	HS	MR	MS	S
Red Bobs	S	HS	MS	R	MR	S
Bozosio	HS	HS	S	MR	MR	S
Progress	HS	HS	HS	HR	HR	MR
Little Club	MS	HS	MS	HS	HR	HR
Pentad	HS	S	HS	MR	S	MR
Emmer	S	HS	HS	R	R	R
Preston	S	MS	S	HR	S	MS
Marquis	HS	MR	MS	MR	MS	HR
Reliance	S	HS	MS	R	MR	R
Garnet	HR	S	R	HR	HR	HR
Hope	R	R	HR	HR	HR	HR

Little Club is highly susceptible to forms 2 and 4, moderately susceptible to forms 1 and 3, and highly resistant to forms 5 and 6 (Fig. 7). Emmer is highly susceptible to forms 2 and 3, susceptible to form 1, and resistant to forms 4, 5 and 6 (Fig. 8). Garnet is susceptible to form 2, resistant to form 3, and highly resistant to forms 1, 4, 5 and 6 (Fig. 9). Hope is highly resistant to forms 3, 4, 5 and 6, and resistant to forms 1 and 2 (Fig. 10). Progress is highly susceptible to forms 1, 2 and 3, highly resistant to forms 4 and 5, and moderately resistant to form 6 (Fig. 11). Preston is susceptible to forms 1, 3 and 5, moderately susceptible to forms 2 and 6, and highly resistant to form 4 (Fig. 12). Pentad is highly susceptible to forms 1 and 3, susceptible to forms 2 and 5, and moderately resistant to forms 4 and 6 (Fig. 13).

Forms 1 and 3 are the only collections the reactions of which do not differ on one or more of the host varieties by at least two classes. Since form 1 is *T. tritici* and form 3 is *T. levis* they can still be considered as different forms on the basis of morphology. The similarity in reaction between these two collections may be due in part to the fact that form 1 showed some *T. levis* spores which in all probability were not unlike those of form 3 in pathogenicity.

Differences in infection percentages at varying temperatures is another basis upon which some of the physiologic forms might be differentiated. From the data presented in Table III it will be seen that form 3 is practically as virulent at 10° C. as forms 1 and 2, especially on the two susceptible varieties Kota and Red Bobs. At 15° C. forms 1 and 2 are still fairly virulent, while form 3

appears to be very non-virulent. Form 3 from Reliance appears to be only weakly parasitic at 15° C.

It is interesting to note that certain of the varieties are more susceptible at one temperature than at another. Progress was equally susceptible to forms 1 and 2 at 10° C., the infection percentages being 81 and 86 respectively. At 15° C. the infection percentages were 0 and 43 respectively for forms 1 and 2. Red Bobs reacted approximately the same at both temperatures with both bunt forms.

Several investigators have reported that in general common wheats are more susceptible to *T. levis*, and the durum wheats are susceptible to *T. tritici*. In these experiments (Fig. 13) 69% of the Pentad (durum) plants were infected with form 1 (*T. tritici*). This same form was also very virulent in most of the common wheats. Forms 2, 3 and 5 (*T. levis*) infected Pentad heavily but not quite to the same degree as form 1. The infection percentages were 54, 64 and 48 respectively. It would appear from these results that in general the common wheats are more frequently infected with *T. levis* but that forms of this species are not necessarily less virulent on the durum wheats than forms of *T. tritici*.

The existence of several physiologic forms differing in their pathogenicity emphasizes the need for a consideration of this phase in the problem of breeding for resistance to bunt. The mere enumeration or classification of numerous physiologic forms is of no particular importance or value to the plant breeder. However, exact knowledge regarding the genetics of bunt resistance can be obtained only from studies with known physiologic forms and pure hosts. It is important therefore to use definite forms whose capacity for infecting parental varieties is fully known. The material just presented is preliminary work leading to an attack upon the wheat bunt problem.

Breeding

Since the breeding studies reported herein are only of a preliminary nature no extensive review of the literature on this phase of the problem seems necessary at this time. A very complete and extensive review was given by Gaines (19) in 1923 and has been well supplemented recently by Briggs (4) in 1926, and again in 1930 (6).

Farrer (13, 14, 15) in Australia as early as 1901 began work on the development of bunt resistant varieties by hybridization. His plan was to subject F_2 and F_3 plants to a heavy attack by bunt and then isolate the resistant selections. The work of Farrer was carried on by his assistants after his death in 1906. This work resulted in the production of two resistant varieties now known as Florence and Genoa.

Little work was done on the breeding of wheats resistant to bunt outside of Australia until 1915, when Gaines (18) began his studies in the United States. In addition to the production of resistant varieties Gaines made a considerable contribution to the knowledge on the genetics of bunt resistance. He crossed varieties possessing varying degrees of resistance to bunt. There were accumulative effects in some of the hybrids and he concluded that multiple genetic factors governed the reaction of the host to the pathogene.

With the discovery and demonstration of the existence of several physiologic forms in the bunt organism additional complications have arisen in the study of the genetics of bunt reaction. This condition necessitates the use of known physiologic forms of the causal organisms in genetic studies. Briggs (4, 5, 6, 7, 8) working in California has made considerable progress in this direction. He has demonstrated the existence of at least two independently inherited dominant factors for resistance. The isolation of the different factors for resistance in the commonly grown varieties of wheat, especially on those varieties to be used as parents in crosses, will be of considerable assistance in the production of varieties resistant to all known pathogenic forms of the causal organism. While detailed knowledge regarding the mode of inheritance of reaction to bunt will be of great help in planning an intelligent attack on the problem, still its lack should not inhibit the initiation of a breeding program in which the hybrid material is inoculated with composite collections of spores from regions in which the improved variety is to be grown.

The breeding studies were initiated for the purpose of improving the commonly grown wheat varieties in their abilities to resist attacks by pathogenic organisms; to withstand unfavorable climatic conditions during the normal growing season, and to improve the quality of the grain, especially when grown on the marginal wooded soils that are lacking in nitrogen. The various hybrid lines and parental varieties are exposed in the segregating generations to as many of these factors as the limited quantity of seed will permit. This is done by making replicate plantings in the different environments and in artificially produced disease epidemics.

When breeding for bunt resistance there are two good reasons for not infecting the F_1 and F_2 plants. The first is that if the plants are susceptible and infection is successful the plants fail to produce seed and are lost for study in later generations. The second is that some susceptible plants nearly always escape infection and their true reaction either is not determined, or their progeny will have to be tested in the succeeding generation. It is more feasible to classify the F_2 plants on the basis of the infection percentages of the F_3 lines. Naturally a limited amount of the crossed and F_1 seed could be inoculated when an indication of the genetic factors operating in the earlier generations is desired. Unless conditions are particularly favorable for infection such that practically all susceptible plants become infected, the ratios of susceptible and resistant plants would not be as reliable as those obtained from F_3 lines.

In these studies the seed produced by the F_2 plants was thoroughly inoculated with chlamydospores from composite collections similar to those used in the varietal test. The seed was sown by hand in five-foot rows one foot apart in 50, 75 or 100 seed plots. The seeding was done when the soil temperature was approximately 10° C. Parental check rows treated similarly to the hybrid material were sown every 30 plots. The field had been summerfallowed in the previous season, so that it would be in good tilth with sufficient moisture for prompt germination, and free from volunteer wheat. At harvest time the

plants were pulled and separated into two classes, bunted and bunt-free. A plant was classified as bunted if it showed any infection whatever. The percentage infection was then calculated from the data thus recorded.

Nine varieties of common wheat were used as parents in this breeding study. Two varieties, Garnet and Reward, were used primarily because of their early maturity and general adaptability to northern regions. Both varieties are able to produce grain of good appearance on poor soils. Reward is superior in baking quality to Garnet and most other varieties commonly grown in western Canada. H_{35} and H_{44} are hybrid selections from a Marquis × Emmer cross made by McFadden (31) in the United States. They are resistant to stem rust and have shown some resistance to bunt at other places, but are not of especially good quality or yielding ability.

The other five parental varieties are pure line selections from crosses between Marquillo (Marquis × Iumillo) and several Marquis × Kanred hybrid selections. Because of the manner in which they originated they are usually referred to as Double Crosses (D.C.). They rank high in yield and quality and have shown considerable resistance to foot-rots and stem and leaf rusts. One of them, I-28-46, has also shown moderate resistance to bunt. These hybrids originated at St. Paul, Minnesota, in the co-operative wheat breeding projects between the Division of Cereal Crops and Diseases of the United States Department of Agriculture, and the Sections of Plant Breeding and Plant Pathology of the University of Minnesota, with which the writer was formerly connected.

The percentages of bunt infection of the parental varieties and the variability in the different check rows are shown in Table V.

TABLE V

PERCENTAGE OF BUNTED PLANTS IN REPLICATED PLOTS OF NINE VARIETIES OF WHEAT INOCULATED ARTIFICIALLY WITH A COMPOSITE CULTURE OF *Tilletia*

Variety	Total number of plots	Number of plots for different infection percentages										
		0	5	15	25	35	45	55	65	75	85	Average
Garnet	30	1	6	8	9	3	2	—	1	—	—	22
D.C. I-28-46	14	1	1	2	3	7	—	—	—	—	—	27
H_{35}	19	—	2	4	5	5	—	3	—	—	—	28
H_{44}	13	—	—	—	4	2	6	1	—	—	—	39
D.C. I-28-125	11	—	—	1	1	2	2	4	1	—	—	43
D.C. I-28-124	23	—	—	—	1	5	6	7	2	2	—	49
D.C. I-28-62	14	—	—	1	—	3	1	5	4	—	—	51
D.C. I-28-60	13	—	—	—	—	2	4	1	2	2	2	59
Reward	13	—	—	1	—	1	1	3	2	4	1	63

Three varieties, namely, Garnet, D.C. I-28-46 and H_{35} are moderately resistant to bunt and the balance are moderately susceptible or susceptible. There is a great deal of variability in the infection percentages as is illustrated in the frequency distribution in Table V. While these varieties are pure lines for all ordinary morphological characters there was no absolute certainty that they were pure lines as far as bunt reaction is concerned. When the seed

was prepared for the parental check plots several check plots of each variety were sown with seed from the same individual plant. The variability within these replicated plots sown with seed from individual plants could then be compared with that of bulk seed of pure lines. The percentages of bunt infection in replicated plots from individual plants are shown in Table VI.

TABLE VI

PERCENTAGE OF BUNTED PLANTS IN REPLICATED PLOTS SOWN WITH SEED FROM INDIVIDUAL PLANTS OF NINE VARIETIES OF WHEAT INOCULATED WITH A COMPOSITE CULTURE OF *Tilletia*

Variety	Plant number	No. of plots	Number of plots for different infection percentages										
			0	5	15	25	35	45	55	65	75	85	Average
Garnet	193	6	—	1	1	2	1	—	—	1	—	—	28
	199	5	1	2	—	1	—	1	—	—	—	—	18
	258	7	—	2	3	1	—	1	—	—	—	—	19
	249	4	—	—	1	2	1	—	—	—	—	—	25
	276	4	—	—	2	2	—	—	—	—	—	—	22
	284	2	1	—	—	—	1	—	—	—	—	—	20
D.C. I–28–46	238	4	—	1	—	1	2	—	—	—	—	—	26
	257	8	1	—	1	1	5	—	—	—	—	—	30
H_{35}	181	5	—	1	1	2	1	—	—	—	—	—	20
	184	6	—	1	—	2	2	—	1	—	—	—	29
	187	5	—	—	1	1	1	—	2	—	—	—	37
	190	2	—	—	2	—	—	—	—	—	—	—	18
H_{44}	155	6	—	—	—	1	1	3	1	—	—	—	43
	158	7	—	—	—	3	1	3	—	—	—	—	36
D.C. I–28–125	172	8	—	—	1	1	1	—	4	1	—	—	44
	177	3	—	—	—	—	1	2	—	—	—	—	42
D.C. I–28–124	154	7	—	—	—	—	2	1	1	1	2	—	55
	157	7	—	—	—	1	2	1	3	—	—	—	44
	186	4	—	—	—	—	1	1	1	1	—	—	44
	189	2	—	—	—	—	—	1	1	—	—	—	30
D.C. I–28–62	192	3	—	—	—	—	—	—	2	1	—	—	58
	198	4	—	—	1	—	—	—	1	2	—	—	50
	219	7	—	—	—	—	3	1	2	1	—	—	48
D.C. I–28–60	275	10	—	—	—	—	1	3	1	2	1	2	61
Reward	220	7	—	—	1	—	1	1	—	1	2	1	61
	259	2	—	—	—	—	—	—	1	1	—	—	61
	324	3	—	—	—	—	—	—	2	—	1	—	64

From a study of the data presented in Tables V and VI it is evident that in general the variability in bunt infection on different plots sown with seed from the same plant is as great as the variability in infection from seed of different plants of the same variety. The variations in infection then can readily be attributed to differences in environmental conditions and the chance of infection with the limited number of individuals used in plant rows, rather than to any inherent differences in the individual plants within each variety.

Large F_3 populations were tested for their reactions to bunt and the average infection percentage of each line was used as an index of resistance or susceptibility. Approximately 150,000 plants were grown and studied in the nine different crosses. The data have been summarized and the results are presented in Table VII.

TABLE VII

PERCENTAGE OF BUNTED PLANTS IN PARENT VARIETIES AND F_3 LINES WHEN INOCULATED ARTIFICIALLY WITH A COMPOSITE CULTURE OF *Tilletia*

Variety or cross	Total number of plots or F_3 lines	Number of plots for different infection percentages										
		0	5	15	25	35	45	55	65	75	85	95	Average
Reward	7	—	—	1	—	1	1	—	1	2	1	—	61
D.C. I–28–62	7	—	—	—	—	3	1	2	1	—	—	—	48
Reward × I–28–62	91	—	—	2	3	9	14	21	19	20	3	—	58
Reward	3	—	—	—	—	—	—	2	—	1	—	—	64
D.C. I–28–60	2	—	—	—	—	1	1	—	—	—	—	—	42
Reward × I–28–60	71	—	—	2	1	8	12	14	14	16	4	—	58
D.C. I–28–124	14	—	—	—	1	4	2	4	1	2	—	—	49
H_{44}	13	—	—	—	4	2	6	1	—	—	—	—	39
I–28–124 × H_{44}	260	—	3	9	34	45	62	48	34	14	8	3	48
Reward	3	—	—	—	—	—	—	1	1	1	—	—	67
D.C. I–28–46	5	—	1	1	2	1	—	—	—	—	—	—	22
Reward × I–28–46	97	—	2	2	2	5	21	23	24	12	3	3	46
D.C. I–28–60	11	—	—	—	—	1	3	1	2	2	2	—	62
Garnet	11	—	1	3	5	2	—	—	—	—	—	—	22
I–28–60 × Garnet	264	8	31	68	46	39	40	21	6	4	—	1	30
D.C. I–28–62	7	—	—	1	—	—	—	3	3	—	—	—	53
Garnet	11	1	3	1	3	1	1	—	1	—	—	—	24
I–28–62 × Garnet	200	3	9	20	35	43	45	23	18	4	—	—	38
D.C. I–28–124	9	—	—	—	—	1	4	3	1	—	—	—	50
H_{35}	9	—	—	4	1	2	—	2	—	—	—	—	30
I–28–124 × H_{35}	140	—	10	10	24	26	31	22	10	5	2	—	39
D.C. I–28–125	11	—	—	1	1	2	2	4	1	—	—	—	43
H_{35}	10	—	2	—	4	3	—	1	—	—	—	—	26
I–28–125 × H_{35}	277	1	20	40	62	56	53	25	26	4	—	—	33
D.C. I–28–46	9	1	—	1	1	6	—	—	—	—	—	—	29
Garnet	8	—	2	4	1	—	1	—	—	—	—	—	19
I–28–46 × Garnet	136	4	5	24	25	28	22	15	8	4	1	—	35

From a study of the distributions in Table VII it will be noticed that in every cross a number of F_3 lines transgressed beyond the range shown by both parents. This may be due in part to the greater number of individual hybrid plots than parental plots, but it is rather indicative also that several genetic factors govern the reaction to bunt and that numerous recombinations have been produced.

For convenience the parent and hybrid populations may be grouped into five major categories on the basis of reaction to bunt. The first two crosses in Table VII are susceptible × susceptible; the third cross, moderately susceptible × moderately susceptible; the fourth, fifth and sixth crosses, susceptible × moderately resistant; the seventh and eighth crosses, moderately susceptible × moderately resistant; and the ninth cross, moderately resistant × moderately resistant. In every cross but one the average infection percentage of the F_3 hybrid lines was intermediate between those of the two parents. In the ninth cross, between Garnet and D.C. I-28-46, both moderately resistant, the average infection percentages are 19 and 29 respectively for the two parents and 35 for the 163 F_3 hybrid lines. In this cross there is a very decided transgressive segregation toward a greater susceptibility.

There is a great similarity between the distributions of F_3 lines originating from different F_2 populations within the same cross. There were three F_2 families in the cross between D.C. I-28-125 and H_{35} from which 277 F_3 lines were grown, and two F_3 families in the cross between D.C. I-28-124 and H_{44} from which 260 F_3 lines were grown. The distributions of the F_3 lines for each F_2 population in these two crosses are shown in detail in Table VIII. In general this same degree of similarity between different F_2 families was present in all of the crosses studied. They are so similar that one would be justified in concluding that the parental material must have been homozygous for the genetic factors governing the reaction to bunt.

TABLE VIII

DISTRIBUTION OF PERCENTAGE BUNTED PLANTS IN F_3 LINES ORIGINATING FROM SEPARATE F_2 FAMILIES WHEN INOCULATED ARTIFICIALLY WITH A COMPOSITE CULTURE OF *Tilletia*

F_2 family	Total no. of F_3 lines	Number of F_3 lines for different[1] infection percentages											
		0	5	15	25	35	45	55	65	75	85	95	Average
D.C. I–28–125 × H_{35}, No. 174	117	—	7	17	17	27	25	12	11	1	—	—	37
D.C. I–28–125 × H_{35}, No. 175	35	—	2	7	10	8	5	1	1	1	—	—	29
D.C. I–28–125 × H_{35}, No. 176	125	1	11	16	35	21	23	12	4	2	—		33
D.C. I–28–124 × H_{44}, No. 156	138	—	2	4	20	17	32	29	20	8	4	2	48
D.C. I–28–124 × H_{44}, No. 159	122	—	1	5	14	28	30	19	14	6	4	1	47

Discussion

Crop improvement programs are too often limited to the consideration of one or two characters which at the moment are of major importance. When a characteristic that may assume the role of a limiting factor in production has been disregarded in a breeding project, the improved variety is likely at any time to be a failure as far as the grower is concerned. This applies particularly to the problem of breeding for resistance to the many destructive diseases common to our field crops. It also applies to such important agronomic and commercial characters in cereals as stiffness of straw, time of maturity, shattering, milling and baking quality, etc. The need for superiority in these

characters is more generally appreciated and sought after in general agronomic breeding programs than in disease reactions. Ceres wheat is a variety that was produced and distributed for its resistance to black stem rust. Later it was found to be highly susceptible to leaf rust, bunt, loose smut, scab and foot-rot. Its predecessor, Kota, was equally undesirable, but was also of inferior milling and baking quality. One of the more recently distributed new varieties, Reward, is very desirable in fulfilling the needs of short season regions. It is also desirable in most other agronomic and commercial characteristics. It is equal, if not superior in baking quality, to Marquis, our present standard variety. It is, unfortunately, very susceptible to bunt. In the tests reported herein 88% of the plants were affected with bunt. Owing to the fact that it is a new variety not many complaints have, as yet, been received from the growers. Bunt is an accumulative disease, however, and in time this weakness will become evident in the field, unless efficient and consistent seed treatment is practised.

Garnet is another early variety which was distributed to fill the same needs as that supplied by Reward. Instead of being equal to Marquis it is inferior, especially when grown on poor or marginal soils, although the appearance may be good (30). It is, however, fairly resistant to bunt. In the same tests with Reward it had only 12% of the plants infected. It was the most resistant of all of the commonly grown varieties tested.

At this stage in the development of crop improvement work it is hardly to be expected that the new productions will be perfect in all respects. Some varieties are highly desirable, even though they possess one or two weaknesses. The important thing is to have some knowledge regarding the reaction of new varieties to the more important limiting factors before they are distributed. It is not fair to the grower to be advised to grow a new variety and then have him proceed to discover its disadvantages or weaknesses. If the information were available first the new variety could be distributed with instructions regarding any necessary precautions for its proper handling. Reward wheat is highly desirable in certain sections of the country, but it should be treated for bunt regularly if the seed is infected or in danger of becoming contaminated.

Once a variety is developed resistant to any particular disease, every precaution should be taken to avoid possible contamination by introducing new physiologic forms from other regions. Heald and Gaines (24) and Holton (27) have suggested that for this reason it would seem important to continue the treatment of all seed, especially new varieties of wheat for bunt. This procedure would lessen to some extent the value and importance of resistant varieties to the grower, since the cost of seed treatment, when considered on the basis of 20 to 25 million acres annually in Canada, is an important item. Naturally the more important advantages are the reduction of losses in the field due to lowering of yields and of grade. The possible necessity of treating seed of varieties that are resistant to the pathogenes in the region where they are grown, in order to avoid contamination by new virulent forms from the outside which are likely to be introduced, emphasizes the need for

precaution in introducing cultures of organisms from foreign countries, and the need for strict plant pest quarantine regulations.

Most varieties of field crops are soon contaminated with admixtures of other varieties. Seed treatment would prevent the infection of any susceptible admixtures, even though the variety itself were highly resistant or immune. An infection of 1% will often cover the threshed grain with sufficient smut to cause the whole crop to grade smutty (23). Purity of variety is one of the best precautions against having a normally resistant variety grade smutty.

The origin of physiologic forms has an important relation to the crop improvement program since the stability and permanence of the resistance bred into a new variety will, to a large extent, determine its usefulness to the grower. New forms may be introduced into the hard red spring wheat region from outside regions, or they may arise as a result of mutation or hybridization. New varieties introduced to control other diseases, or for some particular agronomic characteristic, may be heavily attacked by forms already present but unnoticed because of their low virulence on the older and more resistant varieties. The origin or increase of new physiologic forms by any or all of these methods has been demonstrated in a number of phytopathogenic organisms. There is no good reason for not believing that new forms of *T. tritici* and *T. levis* can originate through these same processes.

Flor (16) has demonstrated recently that monosporidial cultures inoculated into wheat seedlings produced no infection. Heterothallism was indicated when infection was obtained only from mixtures of two monosporidial cultures. These results indicate that, unless there are some homothallic forms as yet undiscovered, hybridization is requisite to successful infection. A resistant variety then must be resistant to all the potential combinations brought about by the natural segregation and recombination of factors for pathogenicity in the parasites. The importance of the range of virulence of any particular culture, or combination of cultures, and their host relationships comes into view.

New physiologic forms of *T. tritici* and *T. levis* have made their appearance in the hard red spring wheat regions of western Canada and the United States. The number and variability of the forms discovered will depend to a considerable extent upon the intensity and nature of the research program on the bunt problem. With an increase in the number of varieties used as differential hosts and in the number of collections of bunt it is conceivable that an unlimited number of forms could be demonstrated. Apart from demonstrating that great variability does exist in the organism as well as the host the mere demonstration of numerous forms may be of no particular value to the plant breeder. It is important, however, that exact knowledge be obtained as to the range in virulence of the pathogenes and the genetic factors governing the host reactions to them. Information regarding the prevalence and distribution of physiologic forms of bunt in the region for which the crop improvement program is designed is a necessity and basic to a well rounded out plant breeding program for resistance to bunt.

There is no absolute assurance that varieties of wheat which have been developed for resistance to bunt in any particular region will remain resistant indefinitely. New or undiscovered forms may arise within the region, or they may be introduced from the outside. This may be true of practically all plant pathogenes, but it has not prevented the production and successful use of numerous varieties of resistant crop plants in reducing losses from destructive diseases.

The presence of strains of pathogenic organisms with varying potentialities as parasites demonstrates that for unknown periods in the past variability has been brought about by those agencies responsible for the usual evolutionary processes. The opinion is sometimes expressed, or inferred, probably unintentionally, that the discovery of these forms suddenly accelerates their production to such an extent that breeding plants for resistance to disease is a hopeless task. The discovery of physiologic forms of a pathogenic organism does not necessarily intensify, or change, the status of the plant breeding problem. Such knowledge should help in the solution of the problem. The complexity of physiologic specialization and its relation to breeding for disease resistance appears to be an enigma for those who fail to appreciate the usefulness of such knowledge in the production of resistant varieties.

Summary

1. A study has been made of the reaction of numerous spring wheat varieties and hybrid selections to several chlamydospore collections of *Tilletia tritici* and *T. levis*, the causal organisms of bunt in wheat.

2. An increase in the amount of bunt in western Canada can be accounted for in part by the use of certain varieties that are more susceptible to this disease than some of those grown formerly.

3. The spring wheats show all gradations in reaction to bunt varying from apparent immunity to high susceptibility.

4. Composite cultures of chlamydospores grown on a number of wheat varieties were used as inoculum when testing varieties and hybrids for their reaction to bunt. The results obtained indicate that such tests are sufficiently reliable to be of great value in determining the reaction of a variety to bunt.

5. Replication of tests is necessary for an accurate determination of varietal reaction.

6. Durum wheats, as a class, have been reported as less susceptible to *T. levis* and more susceptible to *T. tritici* than the hard red spring wheats. The reverse is the case for the hard red spring wheats. Several new varieties such as Kota, Ceres and Progress are highly susceptible to both species of bunt fungi and consequently may be important in the distribution of both of them.

7. *T. levis* is more frequently found on the common wheats than *T. tritici*, but all forms are not necessarily less virulent on the durum wheats than *T. tritici*.

8. The varieties of spring wheat grown in the prairie provinces may be classified into three groups. First, varieties such as Kota, Ceres, Progress, Red Fife, Red Bobs, Preston and Reward that are highly susceptible. Second,

varieties such as Marquis, Renfrew, Reliance, Huron and Kitchener that are intermediate in susceptibility. Third, varieties such as Garnet and Ruby that are fairly resistant, but not sufficiently so as to make seed treatment unnecessary.

9. Commercial immune varieties of spring wheat are not yet available, but Garnet is the most resistant of any commonly grown.

10. The highest per cent infection was obtained when the inoculated seed was sown in soil with a temperature of approximately 10° C.

11. Control of bunt has been complicated by the existence of physiologic forms of *T. tritici* and *T. levis*. Certain varieties of spring wheats are resistant to some forms and susceptible to others.

12. One physiologic form of *T. tritici* and five of *T. levis* were obtained from six collections of bunt. A considerable number of forms could probably be distinguished with numerous collections and the proper differential hosts.

13. When subject to different temperatures, some physiologic forms appeared to respond differently in infection capabilities.

14. The varieties of wheat used as differential hosts are as follows: *T. vulgare*—Kota, Red Bobs, Bozosio, Progress, Preston, Marquis, Reliance, Garnet and Hope; *T. durum*—Pentad; *T. dicoccum*—White Spring Emmer; and *T. compactum*—Little Club.

15. The isolation and study of relatively pure forms of the organism will be necessary for a study of the genetic factors in the host governing the reaction to this disease.

16. Collections of chlamydospores cultured on pure host material for two or three generations should for all practical purposes be sufficiently homogeneous to be considered a physiologic form.

17. The term "physiologic form" is applied in this paper to a purified chlamydospore collection of bunt capable of producing a definite set of reactions on a given group of wheat varieties.

18. The inheritance of resistance to bunt was studied in the F_3 of crosses of (a) susceptible × susceptible; (b) moderately susceptible × moderately susceptible; (c) susceptible × moderately resistant; (d) moderately susceptible × moderately resistant; and (e) moderately resistant × moderately resistant, varieties of spring wheat.

19. The average infection percentage of the F_3 lines was intermediate between that of the two parents in eight of the nine crosses. In the latter case the average infection percentage of the F_3 lines was greater than that of either parent.

20. Multiple factors, the exact nature of which has not yet been determined, govern the reaction to bunt of wheat.

21. Production of resistant varieties suitable for the prairie provinces of Canada offers a very promising means for reducing the losses due to bunt of wheat.

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ACCESSORY FOOD SUBSTANCES FOR OSMOPHILIC YEASTS

I. A BIOACTIVATOR IN HONEY STIMULATING FERMENTATION[1]

By A. G. Lochhead[2] and Leone Farrell[3]

Abstract

Honey was found to contain an active principle which stimulated fermentation by certain osmophilic yeasts of the genus *Zygosaccharomyces* in synthetic media. The substance is dialyzable, insoluble in ether and acetone, not precipitated by 85% alcohol, resistant to heating in acid solution and non-volatile. The activating effect of honey is impaired by heating in alkaline solution and by prolonged exposure to moderate dry heat. The active principle may be separated into two fractions by selective adsorption by charcoal. The adsorbed fraction, which may be recovered by elution with alcohol, and the unadsorbed fraction are relatively inert alone, the presence of both being necessary for the active stimulation of fermentation.

Introduction

As the result of a number of investigations (2, 4, 6, 7, 8) honey fermentation is now recognized as being due to the action of osmophilic yeasts capable of developing in sugar solutions of concentrations sufficient to suppress the growth of most common types. Such sugar-tolerant yeasts have been isolated, not only from fermented honey, but also from such sources of infection as floral nectar (4), the bodies of bees (9), soil from apiary ground (3) and utensils used for extraction (4).

The belief that normal honey is regularly infected is supported by the results of a study of 191 samples from various parts of Canada (5). In all cases sugar-tolerant yeasts were found, though in widely varying numbers. The tendency to ferment was found to increase with increasing yeast infection, while a study of the chemical composition indicated moisture as the outstanding factor affecting fermentation. While yeast infection and moisture were very important predisposing causes, certain anomalous cases of fermentation with comparatively low, and non-fermentation with relatively high moisture and yeast contents, suggested that other factors were also concerned. In the absence of any further explanation from the chemical analysis, the possible presence in honey of some unidentified factor affecting yeast activity suggested itself. In further support of this hypothesis were observations that little or no fermentation occurred with honey-fermenting yeasts in synthetic dextrose broth, whereas the addition of some "natural" nutrient such as yeast extract, wort, etc., resulted in active fermentation. Since the addition of honey produced a similar effect, a more detailed investigation was made of the factor responsible.

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Effect of Addition of Honey to Synthetic Nutrient Solution

The synthetic nutrient solution used was intended to approximate the composition of honey and was based on the average analysis of 20 American honeys reported by Browne (1). One litre of solution contained: sucrose, 19.0 gm.; dextrin, 15.0 gm.; asparagin, 1.4 gm.; K_2HPO_4, 1.0 gm.; $MgSO_4$, 0.5 gm.; NaCl, 0.1 gm.; $CaCl_2$, 0.1 gm. and malic acid, 1.2 gm. Dextrose was used as the major source of carbohydrate, the amount varying in the preliminary qualitative tests from 30%-60%. This was later standardized to 40% for most of the quantitative experiments. The medium, after sterilization, had a pH value of 4.0-4.2, approximating that of normal honey. For the tests, cultures of honey-fermenting yeasts were used, the inoculum being prepared from agar slope cultures suspended in dextrose broth of similar concentration to that of the nutrient solution employed.

Preliminary Qualitative Tests

Preliminary tests were conducted to note the effects of adding to the basic nutrient solution varying percentages of honey solution of equal sugar concentration. Repeated experiments showed that the addition of honey solution in as small a proportion as 1 cc. to 99 cc. of synthetic solution noticeably shortened the period before the visible onset of fermentation. The outstanding difference in composition between the honey solution and the synthetic nutrient solution is the presence of levulose in the honey, amounting normally to slightly over half of the reducing sugars contained.

To determine whether its content of levulose was responsible for the accelerated fermentation noted with the addition of honey, two sets of experiments were arranged, in one of which honey, and in the other, levulose was added in varying proportions to the basic solution. The amount of levulose added in each case was equal to the levulose contained in the added honey of the corresponding test. Similar tests were made with four honey-fermenting yeasts, cultures J7, E6, D1, M1, and also with a mixture of the four. From the data in Table I, showing the results with culture D1, there is an indication of the presence in honey of some factor hastening the onset of fermentation. This factor does not appear to be levulose.

Quantitative Tests

In the preliminary tests the criterion of activation was the observed time of the onset of gas production. Further experiments, however, were made on a more strictly quantitative basis, for which purpose 300-cc. Erlenmeyer flasks fitted with Alwood fermentation valves were used, each containing 100 cc. of medium. The basic synthetic medium employed was that described above and contained for most tests, 40% dextrose, though a medium containing 60% was used for the first series. Solutions containing different proportions of honey were prepared by adding to the basic solutions varying amounts of a honey solution diluted to contain the same amount of sugar calculated as invert sugar. Thus a medium containing 5% honey solution is one prepared by adding 5 cc. of honey solution to 95 cc. of basic nutrient solution both of equal

TABLE I

EFFECT OF ADDITION OF HONEY AND LEVULOSE UPON FERMENTATION OF SYNTHETIC SOLUTION

Substance added	Onset of fermentation (days), duplicate tubes						
	3	4	5	6	7	8	10
Control	− −	− −	− −	− −	− −	+ +	
1% Honey sol'n.	− −	− −	− −	− −	+ +		
5% Honey sol'n.	− −	+ +					
10% Honey sol'n.	− −	+ +					
20% Honey sol'n.	− −	+ +					
30% Honey sol'n.	− −	+ +					
Levulose of 1% honey sol'n.	− −	− −	− −	− −	− −	− −	+ +
Levulose of 5% honey sol'n.	− −	− −	− −	− −	− −	− −	+ +
Levulose of 10% honey sol'n.	− −	− −	− −	− −	− −	− −	+ +
Levulose of 20% honey sol'n.	− −	− −	− −	− −	− −	+ +	
Levulose of 30% honey sol'n.	− −	− −	− −	− −	− −	− −	+ +

sugar content. The flasks were sterilized by autoclaving and inoculated with a suspension of yeast culture D1, isolated from fermented honey (4) and classified as *Zygosaccharomyces mellis* of Fabian and Quinet (2). The flasks were incubated at 28° C. and weighed at intervals of three to five days, the loss in weight being considered due to CO_2 evolution, after corrections were made for any change in uninoculated controls. The figures given represent averages from duplicate flasks.

The effect of varying percentages of honey solution upon CO_2 production is shown in Tables II and III in which are presented the results from tests with solutions containing respectively 60% and 40% sugar. Fig. 1 and 2 show

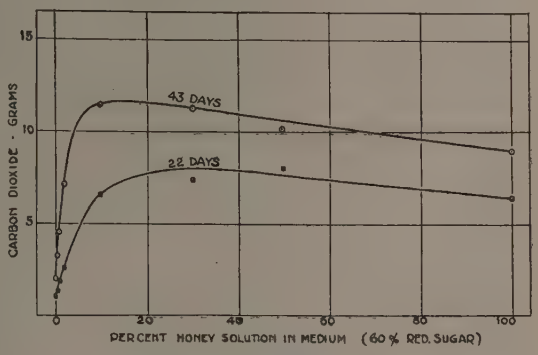

FIG. 1. *Effect of concentration of honey solution on fermentation of synthetic dextrose medium (60% sugar).*

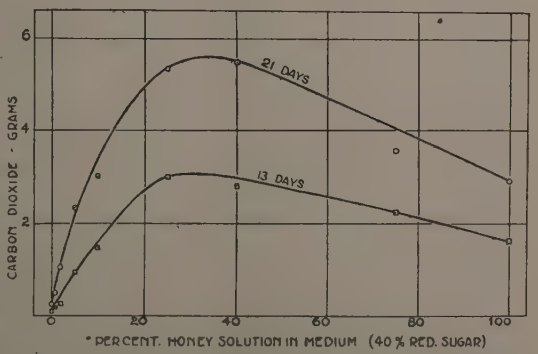

FIG. 2. *Effect of concentration of honey solution on fermentation of synthetic dextrose medium (40% sugar).*

the effect of honey concentration upon total CO_2 production after different periods of time. From these it will be observed that the addition of relatively small proportions of honey has a very marked effect, the curves rising sharply to a maximum and then gradually declining as the nutrient medium approaches a composition of 100% honey solution. To account for this decline two possibilities suggest themselves: the presence of toxic substance in honey or a lack of yeast nutrient materials in honey as compared with the basic solution.

TABLE II

EFFECT OF CONCENTRATION OF HONEY SOLUTION ON FERMENTATION OF SYNTHETIC MEDIUM (60% SUGAR)

% Honey solution	Loss in weight (gm.)										
	5 days	9 days	12 days	19 days	22 days	26 days	29 days	33 days	36 days	40 days	43 days
0	0.25	0.25	0.4	0.65	1.05	1.2	1.1	1.25	1.65	2.0	2.05
0.5	0.05	0.35	0.45	0.9	1.25	1.65	1.75	1.8	2.6	2.95	3.2
1.0	0.2	0.4	0.55	1.35	1.85	2.35	2.55	2.95	3.65	4.25	4.5
2.0	0.2	0.5	0.8	2.0	2.65	3.6	4.2	5.0	5.95	6.75	7.15
10	0.45	1.7	2.35	5.2	6.55	7.95	8.75	9.65	10.5	11.15	11.45
30	0.5	1.75	2.7	5.9	7.3	8.6	9.2	9.8	10.5	10.9	11.2
50	0.35	2.1	3.0	6.9	8.0	8.6	8.95	9.2	9.65	10.1	10.15
100	0.4	2.05	2.8	5.65	6.3	7.25	7.75	7.75	8.45	8.85	8.95

TABLE III

EFFECT OF CONCENTRATION OF HONEY SOLUTION ON FERMENTATION OF SYNTHETIC MEDIUM (40% SUGAR)

% Honey solution	Loss in weight (gm.)						
	3 days	6 days	9 days	11 days	13 days	17 days	21 days
0	0.0	0.05	*0.05*	*0.1*	0.1	0.2	0.25
0.5	0.05	*0.1*	0.1	*0.1*	0.25	0.3	0.55
1	*0.15*	*0.25*	*0.1*	*0.2*	0.15	0.35	0.5
2	0.0	*0.2*	*0.05*	*0.15*	0.25	0.55	1.05
5	*0.15*	*0.2*	0.15	0.4	0.95	1.6	2.35
10	0.0	0.15	0.6	0.9	1.45	2.2	3.0
25	*0.05*	0.35	1.3	1.8	3.0	4.35	5.35
40	0.0	0.25	1.05	1.65	2.8	4.45	5.5
75	0.05	0.3	1.2	1.7	2.25	3.0	3.55
100	*0.15*	0.05	0.06	1.0	1.6	2.25	2.9

NOTE:—*Figures in italics indicate gain in weight.*

This point was investigated in an experiment in which, in addition to the regular series, flasks were prepared with solutions in which extra nutrient salts were added to the dextrose broth-honey mixtures, in such proportions that the total basic nutrient material was the same in each case, and equal to the amount in the basic synthetic solution used as control. Thus when 75 cc. of honey solution was added to 25 dextrose broth, the same amount of extra nutrients was added as in 75 cc. dextrose broth. The results, presented in

Table IV and Fig. 3, lead to the conclusion that the decreasing fermentation noted with the higher proportions of honey in the honey-dextrose broth mixtures is due to a lack of yeast nutrients in the honey which are present in the basic solution chosen. In the case of the solutions with added nutrients (B in Fig. 3) the medium contained, in addition to those added, the nutrients of honey itself, which naturally increased with increasing proportions of honey. This circumstance, however, is not believed to account for the continued rise in curve B up to 100% honey, in view of the results given in Table V and also because the addition of honey ash, as shown later, had no effect in increasing fermentation.

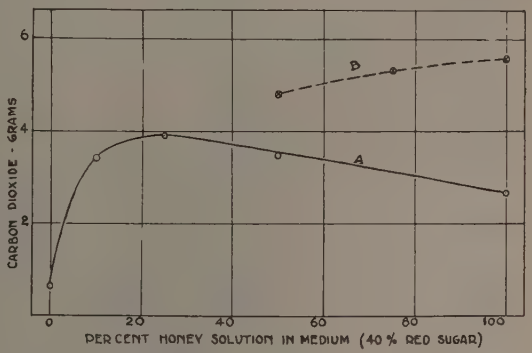

FIG. 3. *Effect of added nutrients on action of honey in stimulating fermentation in synthetic dextrose medium.*

TABLE IV

EFFECT OF ADDED NUTRIENTS ON ACTION OF HONEY

Composition of medium	Loss in weight (gm.)				
	4 days	7 days	10 days	12 days	14 days
Control	0.15	0.05	0.1	0.35	0.65
10% Honey solution	0.4	1.3	2.25	2.85	3.4
25% Honey solution	0.6	1.6	2.65	3.25	3.9
50% Honey solution	0.55	1.35	2.25	2.9	3.45
100% Honey solution	0.45	1.0	1.65	2.05	2.65
50% Honey solution + nutrients	0.75	1.45	3.1	3.95	4.8
75% Honey solution + nutrients	0.9	2.15	3.45	4.4	5.3
100% Honey solution + nutrients	0.7	2.2	3.7	4.75	5.55

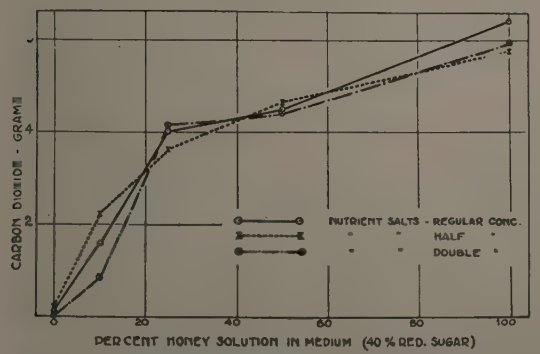

FIG. 4. *Effect of concentration of basic nutrients on action of honey upon fermentation.*

To note the effect of varying the concentration of nutrients chosen for the basic solution, a further experiment was carried out. The influence of various proportions of honey solution added to dextrose broth was noted by preparing different series of solutions in which the concentration of nutrients ranged from one-half to twice that

of the standard medium. Dextrose, sucrose, dextrin and acid remained the same throughout, the mineral salts and asparagin varying as indicated in Table V. The results, summarized in this table and in Fig. 4, indicate that varying the nutrients in the basic medium within the range examined makes little or no difference in the fermentation, and suggest that the basic medium chosen is adequate for the purpose.

TABLE V

EFFECT OF CONCENTRATION OF BASIC NUTRIENTS ON ACTION OF HONEY UPON CONCENTRATION

% Honey	Concentration of basic nutrients	Loss in weight (gm.)				
		5 days	8 days	10 days	12 days	14 days
0% (Control)	×½	0.0	*0.05*	0.05	0.0	0.15
	×1	*0.05*	*0.05*	0.05	0.05	0.1
	×1½	0.0	*0.05*	0.05	0.0	0.1
	×2	*0.05*	0.0	0.05	0.0	0.0
10%	×½	0.05	0.45	0.15	1.75	2.2
	×1	0.05	0.15	0.65	1.10	1.55
	×1½	*0.05*	0.05	0.35	0.65	1.3
	×2	*0.05*	0.05	0.25	0.40	0.8
25%	×½	0.45	1.35	2.2	2.85	3.6
	×1	0.35	1.45	2.45	3.35	4.05
	×1½	0.15	1.10	2.15	3.0	3.7
	×2	0.05	0.9	2.1	3.3	4.15
50%	×½	0.75	2.2	3.25	3.95	4.65
	×1	0.5	2.0	3.05	3.85	4.5
	×1½	0.25	1.7	2.8	3.85	4.35
	×2	0.2	1.3	2.65	3.75	4.45
100%	×½	1.0	2.7	3.95	5.1	5.75
	×1	1.1	3.25	4.8	5.9	6.45
	×1½	0.8	3.15	4.75	5.9	6.4
	×2	0.9	3.1	4.45	5.45	5.95

NOTE:—*Figures in italics indicate gain in weight.*

Characteristics of Bioactivator

With the object of obtaining further knowledge of the substance present in honey stimulating fermentation, additional experiments were made to study the properties of the so-called "bioactivator".

The effect of treatment with adsorbent materials was noted in a preliminary test in which a 90% honey solution was treated with infusorial earth and charcoal* respectively. The honey was boiled with the adsorbent for 10 min., filtered, tubed, sterilized and inoculated with yeasts from fermented honey. It was found that whereas the controls showed fermentation after seven days, the onset of fermentation of honey treated with infusorial earth was delayed but one day, and of that treated with charcoal, five to seven days.

**The source of the charcoal in this case was unknown. Later tests showed that the adsorptive action of different charcoals varied greatly. We noted little or no effect with Merck's animal charcoal, tested on pH ranges from 2.6-7.4, though with Merck's medicinal charcoal, described as blood charcoal, and used for later tests, almost complete inactivation of honey was obtained.*

Effect of Various Treatments

A more extended series of tests was made with a 50% honey solution to note the effect of various methods of handling upon the bioactivator. The activation was observed by adding the treated honey or fractions in 4% proportion to synthetic dextrose solution as previously described, and noting the time of onset of fermentation. All test portions, before addition to the basic solution, were made up to equivalent amounts of original honey. Observations were made of the effect of:

(1) Boiling with medicinal charcoal and fuller's earth.
(2) Dialyzing through parchment membrane.
(3) Drying of pure honey in air at 75° C. for six days.
(4) Autoclaving for one hour at 15 lb. pressure at pH = 4.2 (normal reaction), in neutral solution, and in alkaline solution, final adjustment being made to pH = 4.2.
(5) Distilling.
(6) Treating with lead acetate added to alkaline honey solution, filtering, deleading after filtration and adjusting to pH = 4.2.
(7) Treating with ether, alcohol 95% (to give an 85% solution), and acetone.
(8) Ashing honey.

TABLE VI

EFFECT OF VARIOUS TREATMENTS ON BIOACTIVATOR IN HONEY

Substance added	Onset of fermentation (days)							
	3	4	5	6	7	8	10	13
Control—basic solution	– –	– –	– –	– –	– –	– –	– –	– –
Normal honey	– –	+ +						
Charcoal filtrate	– –	– –	– –	– –	– –	– –	– –	– –
Fuller's earth filtrate	– –	+ +						
Dialysate	– –	+ +						
Air-dried honey	– –	– –	– –	– –	– –	+	+	
Autoclaved honey-acid	– –	– –	+ +					
Autoclaved honey-neutral	– –	– –	– –	+ +				
Autoclaved honey-alkaline	– –	– –	– –	– –	+ +			
Honey distillate	– –	– –	– –	– –	– –	– –	– –	– –
Residue from distillation	– –	– –	+ +					
Lead filtrate	– –	– –	+ +					
Alcohol pp't.	– –	– –	– –	– –	– –	– –	– –	– –
Alcohol filtrate	– –	– –	+ +					
Ether extract	– –	– –	– –	– –	– –	– –	– –	– –
Ether residue	– –	– –	+ +					
Acetone extract	– –	– –	– –	– –	– –	– –	– –	– –
Acetone residue	– –	– –	+ +					
Honey ash	– –	– –	– –	– –	– –	– –	– –	– –

The results, summarized in Table VI, suggest that under the experimental conditions the activating substance in honey is affected by adsorption by charcoal though not by fuller's earth, and is dialyzable. It is insoluble in ether and acetone, though soluble in alcohol (85%). It resists ordinary boiling, is not volatile and withstands autoclaving in acid solution though proving less stable

in alkaline solution. It is noticeably affected, however, by exposure to moderate dry heat. It appears to be absent from honey ash, and is therefore considered to be organic in nature.

Fractionation of Bioactivator

With the apparent adsorption of the activating material by charcoal, attempts were made to recover this by elution with various solvents, for which purpose 1% acetic acid, 1% ammonium hydrate and 95% alcohol were used.

Honey solution of 40% reducing sugar was boiled for 10 min. with 2% medicinal charcoal (Merck), and filtered. The charcoal was removed from the filter and washed with warm water three times. It was then treated with the reagent (100 cc. per 100 cc. of original honey solution) for two hours, after which it was again filtered. In the preliminary trials the filtrate was evaporated to 10 cc. volume, 1 cc. being added to 25 cc. synthetic dextrose solution to note the effect on fermentation. It was observed that although the alcoholic eluate showed a slight activation when added to the basic solution, yet this was much more pronounced when in addition, the solution contained a small amount of the filtrate from the charcoal treatment. The filtrate alone, as previously shown, was relatively inert. It was furthermore noted that the elution with alcohol was much more effective than that with acetic acid or ammonia, and consequently for subsequent tests alcohol was used as standard reagent for elution.

To throw additional light on the apparent existence of two fractions in honey concerned with activation, one of which is adsorbable by charcoal, further quantitative tests were included. Honey was treated with medicinal charcoal as described above and the elution with alcohol similarly conducted. The alcoholic filtrate was evaporated to a few cc. and then made up with distilled water to the same volume as the original honey solution. The single and combined effects of the eluate thus prepared and the charcoal filtrate were compared with that of untreated honey. Additions were made of 10-cc. amounts to the synthetic dextrose solution in Erlenmeyer flasks as previously described, the total volume being 100 cc. The sugar concentration was maintained at 40%.

TABLE VII

EFFECT OF FRACTIONS FROM CHARCOAL TREATMENT

Material added	Loss in weight (gm.)			
	5 days	8 days	11 days	14 days
Control (basic solution)	0.05	0.2	0.35	0.25
Honey (untreated)	0.3	1.45	2.55	3.25
Charcoal filtrate	0.0	0.25	0.35	0.3
Eluate	0.0	0.3	0.3	0.3
Filtrate+eluate	0.2	0.95	1.5	1.85

Results from a typical experiment are outlined in Table VII and Fig. 5, and support the belief that there is in honey a material stimulating fermentation which may be fractionated, giving one portion adsorbable by charcoal and one which is not. Both of these portions appear necessary. From the data it might be inferred, however, that these portions are not different, and that adsorption and elution were very incomplete. In this case filtrate and eluate might contain the same substance, but each in such small quantity as to be ineffective when added alone.

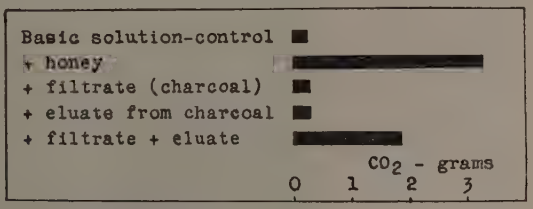

FIG. 5. *Effect of fractions from charcoal treatment on fermentation.*

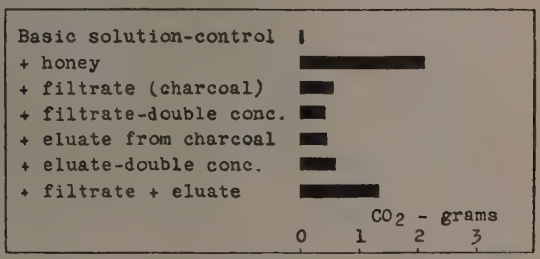

FIG. 6. *Effect of concentration of fractions from charcoal treatment on fermentation.*

With this consideration in view, a series was prepared in which, in addition to the usual amounts, double quantities of filtrate and eluate were also used. The results, presented in Table VIII and Fig. 6, support the belief that the stimulation of fermentation by honey depends upon the presence of at least two active principles. The fact that increasing the amount of either filtrate or eluate is without definite effect leads to the view that the stimulation observed when both filtrate and eluate are present is the result of activating bodies of different kinds rather than a simple additive effect.

TABLE VIII

EFFECT OF CONCENTRATION OF FRACTIONS

Material added	Loss in weight (gm.)			
	4 days	8 days	11 days	14 days
Control (basic solution)	0.0	0.0	*0.1*	0.0
Honey (untreated)	0.25	0.95	1.6	2.1
Charcoal filtrate	0.0	0.15	0.3	0.55
Charcoal filtrate (double)	0.1	0.2	0.3	0.4
Eluate from charcoal	0.1	0.1	0.3	0.45
Eluate (double)	0.1	0.2	0.35	0.6
Filtrate+eluate	0.15	0.65	1.05	1.3

NOTE:—*Figures in italics indicate gain in weight.*

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ACCESSORY FOOD SUBSTANCES FOR OSMOPHILIC YEASTS

II. COMPARISON OF HONEY BIOACTIVATOR WITH BIOS[1]

By Leone Farrell[2] and A. G. Lochhead[3]

Abstract

A bioactivator from honey, stimulating osmophilic yeasts, was compared with Bios. Complementary fractions from treatment with charcoal were found to exert effects similar to Bios I (inosite) and Bios II of Miller and associates, when tested with the Toronto strain of *Saccharomyces cerevisiae*. Charcoal treatment of honey removes by adsorption Bios II leaving a residue, relatively inert by itself, containing inosite. Inosite, however, does not appear as the active substance in the charcoal filtrate for the strain of osmophilic *Zygosaccharomyces* tested, the growth of this organism being dependent upon the presence of another substance which, though not essential for the Toronto yeast, appears to be present in crude Bios II.

Introduction

In a previous communication (6) data were presented indicating the presence in honey of an active principle capable of stimulating fermentation by certain osmophilic yeasts of the genus *Zygosaccharomyces* in synthetic media. The recognition of this bioactivator naturally suggested a possible relationship with accessory factors found by other workers to be necessary for the normal growth and metabolism of certain strains of *Saccharomyces cerevisiae*. Such factors are generally considered under the term "Bios".

Although but thirty years have elapsed since the introduction of the term Bios by Wildiers (12) to denote the substance regarded by him as indispensable to normal yeast development, there has already appeared a voluminous and polemical literature on the subject. A detailed résumé of the work on Bios up to 1925 has been given by Tanner (10), while more recent contributions to the subject have been summarized by the same writer (11) and by Buchanan and Fulmer (1). In addition, a critical survey of the whole Bios question, together with the most pertinent references, has recently been presented by Miller (8). Many of the divergent results of different workers have apparently been due to such causes as the use of ingredients of varying degrees of purity, the employment of different strains of yeast, neglect to prevent contamination by other micro-organisms, etc. Now, however, it is generally agreed that for certain strains of yeast at least, Bios is necessary for development. Other strains, apparently, are able to grow without such accessory substances.

The properties of the substance in honey capable of stimulating the fermentative action of the osmophilic yeasts tested corresponded very closely with those listed by Wildiers. The bioactivator of honey, like Wildiers' Bios, is dialyzable, soluble in water and 80-85% alcohol, but insoluble in ether.

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Contribution from the laboratories of the Division of Bacteriology, Dominion Experimental Farms, Ottawa, with the co-operation of the National Research Council of Canada.

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[3] *Dominion Agricultural Bacteriologist, Department of Agriculture.*

It is resistant to heating in acid solution, although injured in alkaline solution, Since it is not found in honey ash, it is, like Bios, organic in nature. Additional tests showed the honey bioactivator to be non-volatile. Although it is capable of withstanding autoclaving in acid solution (pH = 4.2 approx.), yet prolonged exposure to moderate dry heat (75° C.) noticeably affected it.

From such materials as autolysed yeast and rice polishings a number of workers have isolated definite chemical compounds having an effect upon yeast growth comparable with that of Wildiers' Bios. Thus Eddy, Kerr and Williams (4) described the isolation from yeast of a crystalline compound, $C_5H_{11}NO_3$, of definite melting point, while from rice polishings the isolation of a compound of formula $C_{10}H_8N_4$ with Bios effect has been reported by Kinugasa (5). That the active substance in honey is not similar to these, however, is indicated by the fact that, as previously shown (6), it may be separated into two complementary fractions by treatment with charcoal. The fractions are relatively inert when used alone, though together they noticeably stimulate the fermentation caused by strains of *Zygosaccharomyces*.

Previous workers have reported the fractionation of substances showing Bios activity. From wort and malt combings Lucas (7) succeeded in separating two fractions, called Bios I and Bios II. The former was precipitated while Bios II remained in solution upon treatment with barium hydroxide in alcoholic solution. Neither Bios I nor Bios II alone had any great influence on the growth of the strain of *Saccharomyces cerevisiae* used, though together they greatly increased the yeast crop when added to the basic salts-sugar solution. Bios I has since been identified as inactive inosite by Eastcott (3). The fractionation of yeast extract into two portions by treatment with fuller's earth has been reported by Williams, Wilson and Von der Ahe (15). The fractions obtained showed individually very little Bios activity though a pronounced effect resulted with the two together when used with their strain of yeast. Further work by Williams, Warner and Roehm (14) on fractions derived from yeast extract supported the view that specific strains of yeast vary in their Bios requirements. This point is further emphasized by the work of Copping (2) whose studies indicated that the need for Bios is dependent upon the type of yeast.

In our studies (6) the stimulating effect of honey on fermentation by the strain of *Zygosaccharomyces* used was almost entirely removed by treatment with charcoal. Narayanan (9), however, noted no adsorptive effect with charcoal (norite), using yeast extract as a source of growth stimulant for his strain of *Saccharomyces cerevisiae*. Similarly Williams and Bradway (13), in testing the effect of their fractions on different yeast strains, report that for Wildiers' original yeast culture the essential growth factor present in yeast extract is not readily adsorbed by fuller's earth, and in their data find no evidence of the multiple nature of Wildiers' Bios. In this connection, however, it may be emphasized that fractionation of a yeast stimulant into complementary portions may greatly depend upon the adsorbent used. With our bioactivator from honey we noted little or no adsorptive effect with fuller's earth, kaolin or Merck's animal charcoal, the last-named being employed at

pH values ranging from 2.6 to 7.4. In such cases the filtrate from the treatment was as active in stimulating fermentation as the untreated honey. Using Merck's "medicinal" charcoal, described as blood charcoal, almost complete inactivation of the honey was effected after filtration, and subsequent tests of the unadsorbed and adsorbed fractions permitted the establishment of the multiple nature of the bioactivator.

Comparison of Honey Bioactivator with Lash Miller's Bios

Through the kindness of Prof. W. Lash Miller a series of tests was made possible with the object of comparing the stimulating effect of our bioactivator with that of Bios I and Bios II of the Toronto workers. One set of experiments was conducted in the Toronto laboratory in which were used the standard methods and the strain of yeast employed by the workers there (7). Materials supplied by us consisted of untreated honey solution, filtrate from the charcoal treatment and adsorbed fraction (eluate) recovered by elution of the charcoal with alcohol (6). The results of this experiment as supplied by Dr. Miller are given in Table I and Fig. 1.

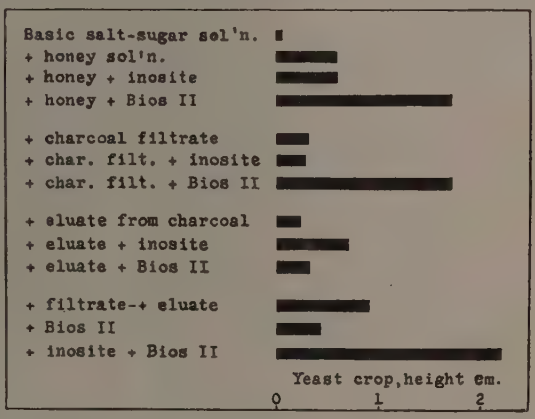

FIG. 1. *Effect of honey bioactivator and Bios upon Saccharomyces cerevisiae (Toronto strain).*

TABLE I

EFFECT OF HONEY BIOACTIVATOR AND BIOS UPON *Saccharomyces cerevisiae* (TORONTO STRAIN)

Substance added	Yeast crop (cm.)	Substance added	Yeast crop (cm.)
Salts-sugar solution (control)	0.05	Eluate from charcoal	0.22
Honey solution	0.57	Eluate+inosite	0.67
Honey+inosite	0.58	Eluate+Bios II	0.32
Honey+Bios II	1.70	Filtrate+eluate	0.88
Charcoal filtrate	0.30	Bios II	0.43
Charcoal filtrate+inosite	0.27	Inosite+Bios II	2.20
Charcoal filtrate+Bios II	1.70		

It will be observed that the bioactivator from honey exerts a Bios effect and that the fractions from the charcoal treatment have a complementary action, characteristic of Bios I and Bios II. The yeast crop from the charcoal filtrate is not increased by adding inosite (Bios I) though noticeably greater

with the addition of Bios II, suggesting that our filtrate contains inosite. With the charcoal eluate, on the other hand, the crop is increased by adding inosite, but not significantly increased with the addition of Bios II. This points to the probable identity of the active substance in our eluate with Bios II, a hypothesis strongly supported by the fact that Bios II is adsorbed by charcoal. It would appear, therefore, that for the Toronto yeast, our filtrate and eluate are qualitatively interchangeable with Bios I and Bios II respectively. The Bios II solution used was apparently much more potent than our charcoal eluate. It may be pointed out, however, that our fractions were purposely diluted to volumes corresponding with the original honey solution.

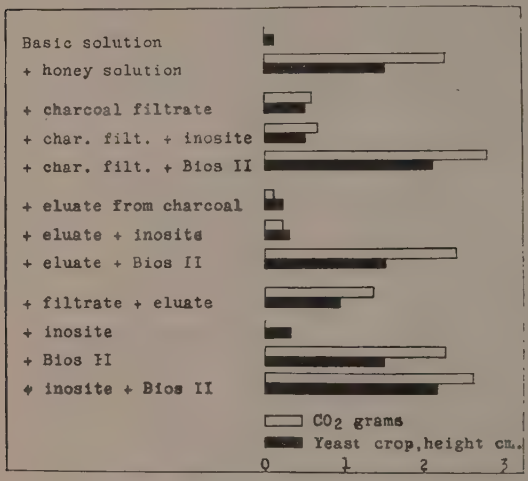

FIG. 2. *Effect of honey bioactivator and Bios upon Zygosaccharomyces mellis.*

Corresponding experiments were conducted in this laboratory with the strain of *Zygosaccharomyces mellis* (Culture D1) isolated from fermented honey, and following the procedure described for earlier tests (6). All solutions contained 40% dextrose, with a 14-day incubation period, previously adopted for osmophilic yeasts. In addition to the CO_2 determinations, estimations of the yeast crop at the end of 14 days were made by measuring the height of the yeast column after centrifuging into a narrow tube 2 cc. of the test solution mixed with 2 cc. of 10% chloracetic acid (7). The inosite and Bios II solution ("crude Bios II") were supplied by Dr. Miller. The results of two separate sets of experiments, each made in duplicate, are shown in Table II and Fig. 2.

From the data it will be seen that the stimulation of the preparations tested was different, with our osmophilic yeast, from the effect produced with the Toronto strain. With our yeast, the yeast crop and CO_2 production with the charcoal filtrate were not significantly increased with inosite, though noticeably so with Bios II. Differing from its effect on the Toronto yeast, however, Bios II, which contains no inosite, has a pronounced effect alone on our yeast. There is thus reason to believe that the growth and fermentative action of our *Zygosaccharomyces* is not dependent on the presence of inosite, which, as the data in Table I indicate, may be contained in the filtrate. This is further supported by its behavior in the presence of inosite and the charcoal eluate. With our yeast no complementary effect was noted, the addition of inosite to eluate having no significant effect over either alone, whereas with the Toronto

TABLE II

EFFECT OF HONEY BIOACTIVATOR AND BIOS UPON *Zygosaccharomyces mellis* (CULTURE D1)

Substance added	Experiment 1		Experiment 2	
	CO_2, gm.	Yeast crop, cm.	CO_2, gm.	Yeast crop, cm.
Basic solution (control)	0.05	0.2	*0.05*	0.05
Honey solution	2.6	1.5	1.9	1.5
Charcoal filtrate	0.55	0.6	0.65	0.4
Charcoal filtrate+inosite	0.7	0.6	0.65	0.4
Charcoal filtrate+Bios II	2.8	2.1	2.8	2.1
Eluate from charcoal	0.2	0.4	0.0	0.05
Eluate+inosite	0.3		0.15	0.3
Eluate+Bios II	2.35	1.45	2.45	1.6
Filtrate+eluate	1.2	0.9	1.5	0.95
Inosite	0.05	0.3	*0.05*	0.35
Bios II	1.75	1.4	2.7	1.6
Inosite+Bios II	2.9	2.55	2.35	1.8

NOTE:— *Figures in italics indicate a gain in weight of flasks.*

yeast, the addition of inosite to eluate resulted in a significant increase in the crop. With our yeast, therefore, inosite is unable to replace the charcoal filtrate as complement to the eluate, further supporting the belief that the active substance contained in our filtrate is not identical with inosite. As inosite and our eluate permit of good growth with the Toronto yeast, this unknown substance is not essential for growth of that strain, though apparently it is present in crude Bios II.

Acknowledgment

The writers wish to express their thanks to Dr. W. Lash Miller of the University of Toronto for having tested their preparations with his strain of yeast and for having kindly furnished them with a supply of Bios I and II.

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THE USE OF THE PROJECTION MICROSCOPE AND PHOTO-ELECTRIC CELL

II. BLOOD STUDIES[1]

By Alfred Savage[2] and J. M. Isa[3]

Abstract

This is a continuation of observations published in this Journal (4). It deals with an electrical photometer of high sensitivity but unaffected by red light. This is used in conjunction with the projection microscope and applied to a study of red blood cells stained with basic fuchsine. The measurements reported are a complex of staining intensity and area. These are compared with area estimations alone and it is shown that the former constitutes a greater variable than the latter. This is particularly noticeable when dealing with anemic blood. The relationship between primary and secondary anemia of man is touched upon.

Introduction

In 1930 Savage and Jamieson (4) published a preliminary note which indicated that by the combined use of the above instruments it was possible to determine the comparative areas of certain microscopic images more quickly and more easily than by other methods. Their observations were confined to direct measurements of the photo-electric current and, in consequence, the degree of magnification which they employed was limited. With the light at their disposal 500 diameters could not be exceeded. This sufficed for the larger microscopic objects but was not enough for work with the elements of animal tissues. Therefore it is evident that this method must undergo extension before it becomes applicable to problems in physiology and pathology.

Scope

The present account begins where the previous note ended and deals with observations made in the same general manner. The essential difference is that, in this instance, the photo-electric current was amplified by means of a thermionic valve and measured indirectly. Increased magnification of the images was possible because the inevitable decrease in light intensity was offset by the amplification employed.

Apparatus

The relative positions of microscope, illuminating device and photo-electric cell have been described (4). Added electrical equipment consisted of a special thermionic valve and the necessary accessories for operating it.

Fig. 1 indicates the circuit. The valve was a "Pliotron FP-54" designed by Metcalf and Thompson (1). It was operated at the voltages recommended and the plate current was balanced out as by Razek and Mulder (3) and others.

[1] *Manuscript received August 8, 1931.*
Contribution from the Manitoba Agricultural College, Winnipeg, Canada, with financial assistance from the National Research Council of Canada.

[2] *Professor of Animal Pathology, Manitoba Agricultural College.*

[3] *Special Assistant, Department of Bacteriology and Animal Pathology, Manitoba Agricultural College.*

The galvanometer was of the d'Arsonval type. It had a sensitivity of 0.003 microamperes and a period of three seconds. An Ayrton shunt was connected with it.

The grid leak consisted of about five feet of fine glass rod bent to a spiral 6 in. long, dipped in India ink and sealed into a glass tube 2 in. in diameter.

An extensive shield of sheet copper enclosed the entire amplifying apparatus.

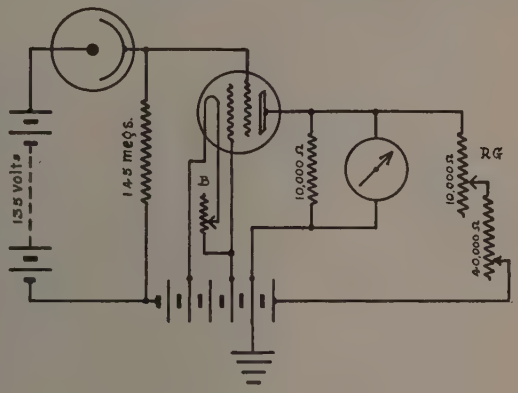

FIG. 1. *Diagram of electrical circuit.*

Operation

When the apparatus was to be used, the current was switched on to both the filament and plant an hour beforehand. Then the photo-electric circuit was closed and the galvanometer balanced at progressive stages until the full current reached it. Finally the lid of the copper shield was closed. Subsequent adjustments to the circuit were made from outside and amounted to very little, the galvanometer "drift" being about 5 cm. per hr.

The lamp for the projection microscope also had to be turned on at least one hour before use. If this were not done the change of its position, due to expansion by heat, appreciably altered the focus and consequently the degree of illumination. It is repeated that this lamp was a spiral filament and therefore not a homogeneous source of light. So far as possible this was overcome by focussing sharply on an incandescent area such as the one indicated in Plate 1, Fig. 1, and by keeping that spot projected into the diaphragm of the photo-cell.

Small changes in the supply current for this lamp (110–115 volts a.c.) were very annoying and could be avoided only by working between the hours of 1 and 5 a.m., when they became negligible.

Behavior of Apparatus

Calibrated with a carbon filament lamp, the photometer had a sensitivity of about 1500 cm. per lumen. The light-current relationship was rectilinear within the range used.

With eosin and fuchsine, it was experimentally verified that dyestuffs in solution transmit light in inverse proportion to the logarithms of their concentration. This is stated because the result of measuring stained objects by this method is a complex product of staining intensity and area. No attempt was made to separate these variables.

Observations on Blood Films

(*1*) *Preparation and Staining*

To be satisfactory for this kind of study, blood films had to be made so that nearly every corpuscle was separated from its neighbors by a distance of several times the diameter of each. This was a somewhat exacting prerequisite in the selection of material.

The films were fixed by immersion in a saturated aqueous solution of mercuric chloride for 20 min. and then thoroughly washed in distilled water. They were stained with acid fuchsine (saturated aqueous solution) for 10 min. and washed as above until no trace of stain could be detected in the plasma between the corpuscles. Rapid drying followed, first by waving the slides to remove excess water, then by placing them in an oven at 54° C.

Projection was at 1400 diameters magnification. The diaphragm of the photo-cell was reduced to a $\frac{3}{4}$-in. aperture as a matter of convenience. From this point, the same procedure was followed as in dealing with rust spores (4), observations being made on 500 erythrocytes from each slide.

(*2*) *Normal Blood*

A film of normal blood gave galvanometer deflections which, on arrangement into classes, occurred with the frequencies given by Table I. The result is a normal curve having 15 classes.

TABLE I

SIZES AND FREQUENCY DISTRIBUTION OF NORMAL RED BLOOD CELLS AT 1400 DIAMETERS AS INDICATED BY THE PHOTO-ELECTRIC CELL

Deflection of galvanometer in mm.	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Frequency	3	2	6	22	45	78	75	104	70	44	25	12	7	6	1

The diameters of 500 corpuscles were then taken at 3000 diameters (by actual measurement of the projected images) and recorded to the nearest 0.5 mm. Similarly arranged, these figures are given in Table II. In this case also, the result is a normal curve, the functions of which are close to those given by Price-Jones (2, p. 10) for measurements made in the same way.

TABLE II

DIAMETERS AND FREQUENCY DISTRIBUTION OF NORMAL RED BLOOD CELLS AS AT 3000 × MAGNIFICATION

Diameter in mm.	17		18		19		20		21		22		23		24		25		26		27
Frequency		1		1	0	2	3	12	13	27	36	49	67	79	63	57	40	31	11	4	2	2

Because of the different number of classes in the two tables given above, they are not strictly comparable in their present form. Even so, one very interesting conclusion may be drawn by comparing them.

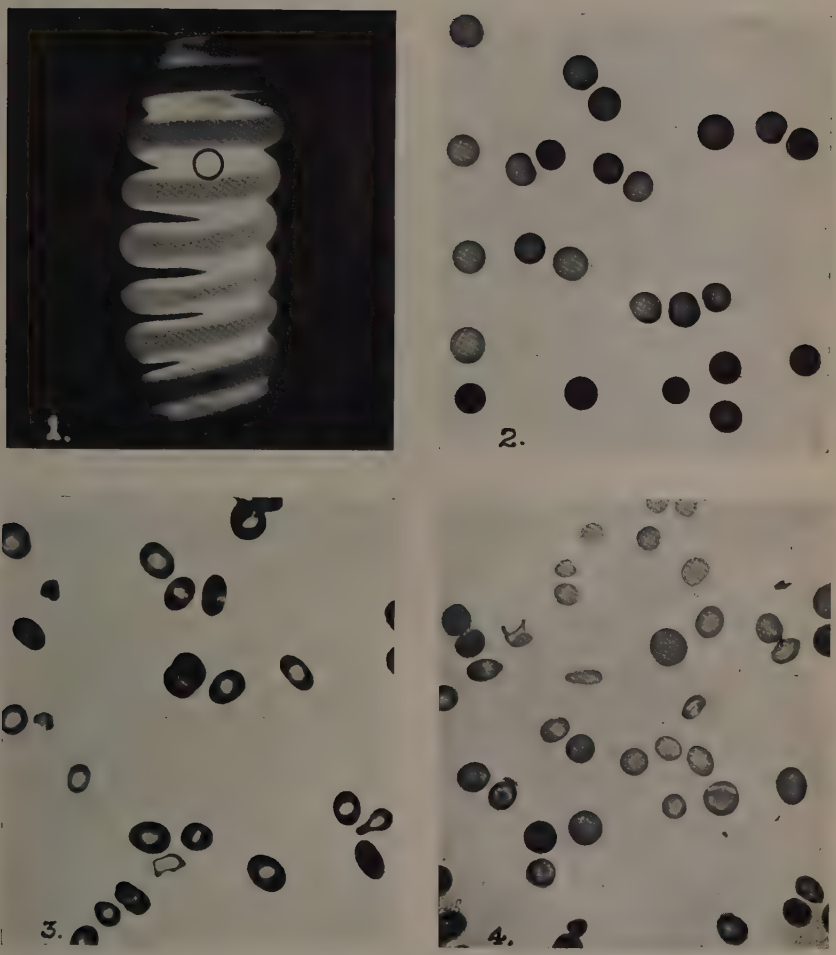

FIG. 1. *Enlarged image of the incandescent filament used as light source. An area similar to that indicated by the small circle was maintained in critical focus.*

FIG. 2. *Normal red blood cells* × *690.*

FIG. 3. *Red blood cells in primary anemia* × *690.*

FIG. 4. *Red blood cells in secondary anemia* × *690.*

Table II deals with diameters of from 17.5 to 27 mm., or varying over a range proportional, roughly, as 1.0 : 1.5. Now the areas of circles vary directly as the squares of their diameters and for this reason one would expect Table I to show an extreme class variation proportional to the squares of these figures, namely as 1 : 2.25. Actually the range is from 6 to 20, or as 1 : 3.3. This is approximately 50% greater than if areas alone were registered by the photo-cell and can be explained only by variations in staining intensity.

(*3*) *Secondary Anemia*

By the photo-electric method, a blood film from a case of secondary anemia (3,700,000 red cells per cu. mm.: hemoglobin, 22%) provided the data in Table III.

TABLE III

SIZES AND FREQUENCY DISTRIBUTION OF 500 RED BLOOD CELLS FROM A CASE OF SECONDARY ANEMIA AS INDICATED BY THE PHOTO-ELECTRIC CELL

Deflection of galvanometer in mm.	1	2	3	4	5	6	7	8	9	10	11	12
Frequency	16	57	97	111	102	54	34	15	6	4	3	1

To complete the basis of comparison used in the instance of normal blood, diameter measurements of the corpuscles were made in the same way. In this instance a difficulty was presented by about 50% of the cells being other than circular. They were not grossly distorted but compelled a selection for measurement of only the round ones. The result (to the nearest mm.) is given as Table IV. In addition, the writers satisfied themselves that, so far as the eye could discern, *there were present neither larger nor smaller cells* than those represented by the extreme figures in this table.

TABLE IV

DIAMETERS AND FREQUENCY DISTRIBUTION OF 500 RED BLOOD CELLS FROM A CASE OF SECONDARY ANEMIA, AS AT 3000 × MAGNIFICATION

Diameter in mm.	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Frequency	3	6	10	25	64	89	93	84	69	33	15	3	2	3	1

A comparison of Tables III and IV shows that the proportional range of the latter is as 1 : 2 (or slightly less): of the former, as 1 : *12!* This surprising difference will be discussed later.

(*4*) *Primary (Pernicious) Anemia*

The blood film selected as representing this condition had been mounted under a coverslip and was somewhat faded. It was therefore unmounted by immersion in xylol, decolorized with acid alcohol and restained in the same manner as were the other slides studied.

Galvanometer readings obtained from this preparation are given as Table V.

TABLE V

SIZES AND FREQUENCY DISTRIBUTION OF 500 RED BLOOD CELLS FROM A CASE OF PERNICIOUS ANEMIA AS INDICATED BY THE PHOTO-ELECTRIC CELL

Deflection of galvanometer in mm.	1	2	3	4	5	6	7	8	9	10	11	12	13
Frequency	16	29	34	57	83	106	98	44	23	3	3	2	2

Owing to the severe and varied distortions of outline presented by the red cells on this slide, it was considered hopeless to attempt estimation of their areas by means of linear measurements.

Discussion

For comparison the three curves obtained photo-electrically are plotted on the same base line and shown as Fig. 2.

These are all apparently normal frequency distribution curves except that B and C end abruptly over the lowest galvanometer deflection which was recorded. This is probably because no attempt was made to read the apparatus to less than 1.0 mm. Even so, two facts seem to have been brought to light. The first is that the complex measured, namely area × staining intensity, of red blood cells varies more than the areas alone. In the case of normal blood this may be presupposed to have certain limits which have not been determined as yet. Possibly the different ages of the corpuscles affords a partial explanation. However, when pathological blood is considered, other factors enter.

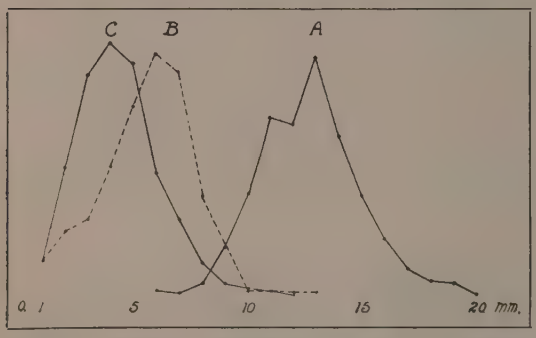

FIG. 2. *Frequency distributions of area × staining intensity of red blood cells in A, normal blood; B, primary anemia and C, secondary anemia as shown by the electrical photometer. 500 cells are represented in each case.*

In ordinary anemia of mild degree, there is a lowering of the hemoglobin content per cell without any noticeable alteration in the size and shape of these elements. This is accompanied by a decreased affinity for the "acid" stains. According to the technique used in these observations the effect of this would be to move the whole curve to the left thus increasing the degree of variation by lowering the value of the mean or, as the writers have chosen to express it, by increasing the proportional spread between the cells of largest and smallest diameter. When the anemia is more marked two other factors

augment this effect. These are: (a) marked variations in the sizes of the cells and, (b) obvious inequalities in their staining reactions. It is suggested that the number of resulting combinations which are possible affords ample explanation for the degree of variation observed.

The second fact concerns a difference between primary and secondary anemia. Evidence is too limited to permit a general conclusion on this point. But, if the slides studied may be considered as typical, it is clear that the above method shows only a difference of degree (which is probably a matter of chance) and not one of kind.

This is in apparent contrast with the findings of Price-Jones (2, p. 10) who studied and measured the red cell diameters in normal blood and in anemias of both types. His figures were arranged to show the frequency distribution of the sizes of these cells in all three conditions. The general conclusion reached by this worker was that the mean red cell diameter is decreased in secondary but *increased in primary anemia*. If to this might be added the fact that in the latter condition the hemoglobin content per cell is usually normal or greater than normal, there would be reason to suppose the above contrast more real than apparent. It is emphasized however that the results are not strictly comparable.

Finally, whether or not the above technique proves of value to the research worker engaged in the study of blood, the writers are of the opinion that no other combination of instruments is capable of giving the same results for analytical and statistical purposes.

Acknowledgments

To Dr. P. A. Macdonald of the Physics Department, University of Manitoba, the writers are indebted for much sound advice regarding the electrical aspects of the problem and for the loan of the Pliotron valve. Dr. S. Meltzer and Dr. D. Nicholson of the Winnipeg General Hospital very kindly supplied the slides of pathological blood. Mr. D. Binnington of the Chemistry Department, Agricultural College, constructed the grid leak.

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SELECTIVE HEATING BY SHORT RADIO WAVES AND ITS APPLICATION TO ELECTROTHERAPY[1]

By J. C. McLennan[2], F.R.S. and A. C. Burton[3], M.A.

Abstract

In the first part of the paper the theoretical basis of the formulas, given in a previous communication, for the generation of heat in a poorly conducting dielectric which is placed in the field of a high-frequency oscillator, is examined in some detail. Its application to the complicated case met with in medical "radiothermy" is made and it is shown that the analysis applies with satisfactory accuracy to this case, though not to that of electrode diathermy. Prediction of the "selective" effect is possible from a knowledge of the characteristic electrical constants of the body-substances at high frequencies, and by proper choice of wave-length the heating of a particular part might be favored over that of neighboring parts.

In the second part of the paper, experimental work is described which carries verification of the formulas to shorter wave-lengths. Experiments on the heating of meat demonstrate the selective effect and its dependence upon wave-length. The heating of such substances as liver, heart, and the different parts of an egg, are examined experimentally as examples of the various determining factors that are involved.

The theoretical explanation of the effects is considered satisfactory and further developments depend upon the results of *in viva* experiments with shorter wave-lengths than those at present in general use.

Important developments in the application of high frequency electric fields to medicine have been made in the last two or three years. The discovery that a body, placed in the "condenser-field" of a high frequency oscillator of wave-length 50 metres and less, was heated by the oscillations, has been applied extensively in the production of artificial fevers in man. The use of ultra-high frequency in electrotherapy promises to be of very great importance.

The results of a research into the physical causes of the heating and its dependence upon the physical character of the body or bodies being heated, as well as upon the frequency and intensity of the field, have been published (6). The amount of heating was shown to be given, to a close approximation, by the formula,

$$\frac{dT}{dt} = \frac{E^2}{\rho s} f(\theta, \epsilon, K) \frac{x}{1+\left(\frac{2x}{K\nu}\right)^2}, \qquad (1)$$

where s = specific heat, ρ = density, x = conductivity in absolute units, K = dielectric constant, and $f(\theta, \epsilon, K)$ is a function of the orientation (θ), the shape (ϵ) and dielectric constant K of the body, whilst E and ν are the intensity and frequency of the high frequency electric field. A paper dealing with the same problem but adopting a rather different method was published at the same time by Pätzold (9), who reached the same general conclusions.

There are two characteristics, peculiar to this method of production of heat, that are significant in its application to medicine. The first is that the heating takes place throughout the interior of the body, the "skin-effect" being

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of negligible importance in this problem. This makes the use of short wave heating, or "radiothermy", a far more effective method of heat treatment than any external application of heat could be. By its means, the temperature of the blood may rapidly be raised and artificial fevers induced, which before could be produced only by the introduction of irritants such as malarial germs.

The second peculiarity is that the heating is not produced uniformly throughout a heterogeneous body; that there is a selective action, predicted by the formula (1) and shown experimentally to exist. In the special case of a body in which the dielectric constant is the same throughout but the conductivity varies, the maximum heat per unit volume per second is produced in a region for which the relation (2) is true.

$$x = \frac{K\nu}{2}. \tag{2}$$

It may be pointed out that, even if the heat were uniformly produced throughout the body, the difference in the specific heats of different parts would make the resultant rise in temperature a maximum in certain places. The distribution of temperature after heating must then be very different from that produced by the flow of heat into the body from an external source, in which case the temperature gradient must always be of the same sign.

Equation (2) shows that in the choice of the frequency, ν, of the oscillator, we have a means of altering at will the distribution of the heating and consequent temperatures throughout the body. In general, the longer waves, of lower frequency, choose the less conducting portions; the higher frequencies select the portions of greater conductivity.

The application of this selective effect, rather than the general heating effect, has not yet been specifically made to medicine, but the possible usefulness is so great that further examination of the theoretical basis, and further experimental verification has been made, and is described in what follows.

Theory

Since Pätzold (9), Pierce (10), and others have dealt with the problem as a "circuit" problem, in the particular cases represented by the experimental conditions respectively adopted, a general, but quite simple, treatment of the problem by this method is now given. The procedure will be from the simple particular cases to the complexity of the actual conditions of practice.

(a) Condenser shunted by a resistance. Referring to the diagram, Fig. 1, for the meaning of the symbols, if V is the instantaneous potential across the condenser,

$$V = I_2 R \qquad I_1 = j\omega c V \qquad I = I_1 + I_2 = V\left[\frac{1}{R} + j\omega c\right]$$

$$I^2 = V^2\left[\frac{1}{R^2} + \omega^2 c^2\right],$$

and there is a phase difference between I and V given by

$$Cos\ \theta = \frac{1}{\left[1 + \omega^2 c^2 R^2\right]^{\frac{1}{2}}}.$$

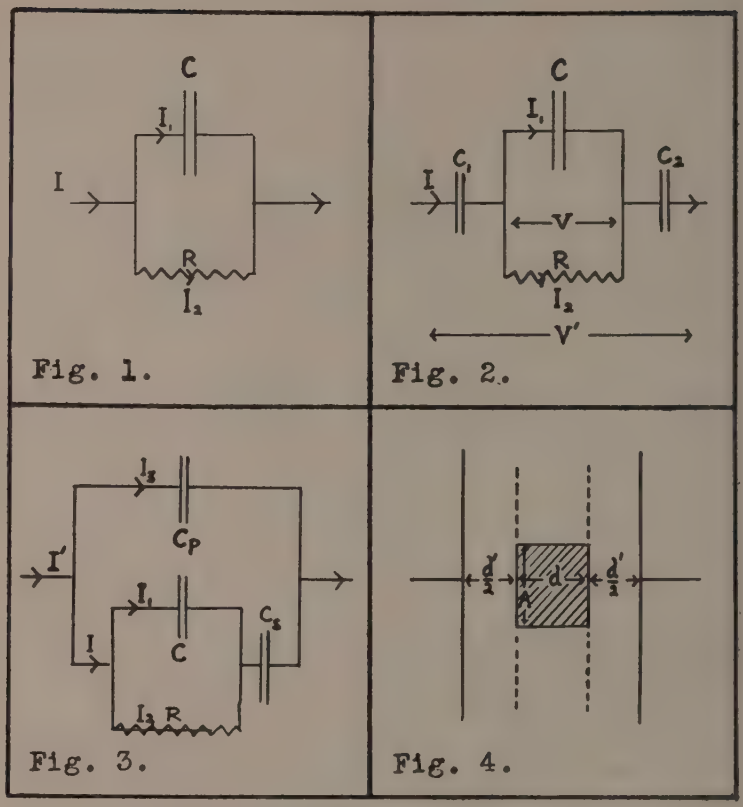


FIG. 1, 2, 3 and 4. *'Equivalent circuits'.*

Power loss
$$P = VI\cos\theta = \frac{V^2}{R}, \tag{3}$$

or
$$P = I^2 \cdot \frac{\frac{1}{R}}{\left[\frac{1}{R^2} + \omega^2 C^2\right]} = I^2 \cdot \frac{R}{1+\omega^2 C^2 R^2}. \tag{4}$$

In any experiment, we may arrange, as we alter the value of R, by, for instance, replacing the solution in the condenser by another of different conductivity, that either the potential V or the total current I is kept constant. In the first case, V being constant, the maximum heating is when R is least. In the second case, differentiating in (4) we get for the maximum heating the relation,

$$R = \frac{1}{\omega C}. \tag{5}$$

The latter is one of the cases dealt with by Pätzold.

(b) Suppose now that the circuit is as in Fig. 2, capacities C_1 and C_2 being added in series with the "leaky" condenser. This is the equivalent circuit of the case where the electrodes are not in contact with the solution

but placed outside the cell containing it. Let V' now be the total drop of potential between the condenser plates of the oscillator.

As in (a)

$$V = \frac{I}{\frac{1}{R} + j\omega c}.$$

There is a potential drop across the condenser C_1 given by $V_1 = \frac{I}{j\omega c_1}$, and across C_2 given by $V_2 = \frac{I}{j\omega c_2}$.

Then $V' = V_1 + V_2 + V = I\left[\frac{1}{j\omega}\left(\frac{1}{c_1} + \frac{1}{c_2}\right) + \frac{1}{\frac{1}{R} + j\omega c}\right]$.

Let $\frac{1}{c_1} + \frac{1}{c_2} = \frac{1}{c_s}$, c_s being the total capacity in series with the "leaky" condenser.

Then

$$I = \frac{V'}{\frac{1}{j\omega c_s} + \frac{1}{\frac{1}{R} + j\omega c}}.$$

Rationalizing, and making use of the convenient abbreviations $\frac{c_s}{c} = a$ and $\frac{1}{R\omega c} = h$, we find that the real component of I, that is, the component in phase with V, is:—

$$I_R = \frac{V'\omega c h}{(1+a)^2 + a^2h^2},$$

and the power loss is therefore:—

$$P = V'I_R = \frac{(V')^2\omega c h}{(1+a)^2 + a^2h^2}$$

$$= (V')^2 \frac{R}{R^2\left(1 + \frac{c}{c_s}\right)^2 + \frac{1}{\omega^2 c_s^2}}. \qquad (6)$$

Since there is no added loss in the capacity C_s, the power loss in terms of the current I is, as before,

$$P = I^2 \frac{R}{1 + \omega^2 c^2 R^2}.$$

Examination of these equations as R varies shows that there are now maxima in each of the two cases, that of constant voltage and that of constant current. The values of R for maximum heating are respectively

$$\text{Constant voltage:} - \quad R = \frac{1}{\omega(c + c_s)}. \qquad (7)$$

$$\text{Constant current:} - \quad R = \frac{1}{\omega c}, \text{ as before.}$$

(c) If the area of the condenser plates is greater than the cross section of the liquid in the cell, there will be in addition to the capacities already considered, a capacity C_p between the condenser plates in parallel with the circuit of (b). There is an additional current through C_p,

$$I_3 = j\omega C_p V'.$$

There is, however, no additional power loss here, so that the total heating is still given by (6),

$$P=(V')^2\frac{R}{R^2\left(1+\frac{c}{c_s}\right)^2+\frac{1}{\omega^2c_s^2}}.$$

The relation between the total current I' and the potential is now changed.

$$I'=I+I_s=V'\left[\frac{1}{\frac{1}{j\omega c_s}+\frac{1}{\frac{1}{R}+j\omega c}}+\frac{1}{\frac{1}{j\omega c_p}}\right].$$

This gives for the power loss:—

$$P=(I')^2\frac{R}{\left(1+\frac{c_p}{c_s}\right)^2+\omega^2R^2\left[c\left(1+\frac{c_p}{c_s}\right)+c_p\right]^2}. \tag{8}$$

The conditions for maximum heating are then:—

Constant voltage:— $R=\frac{1}{\omega(c+c_s)}$, as in (7).

Constant current:— $$R=\frac{1}{\omega\left(c+\frac{c_pc_s}{c_p+c_s}\right)}. \tag{9}$$

In our experiments, the voltage was constant, and Equation (7) applied; in Pätzold's experiments the total current I' was kept constant and Equation (9) was applicable.

In either case, if the ratio $\frac{c_s}{c}$ is small compared to unity, the formula for the power loss becomes,

$$P=V^2c_s^2\frac{\omega^2R}{1+\omega^2c^2R^2}=(I')^2\left(\frac{c_s}{c_p+c_s}\right)^2\frac{R}{1+\omega^2c^2R^2}, \tag{10}$$

and whether current or voltage is regarded as constant, the maximum is when $R=\frac{1}{\omega c}$ approximately. This was the law proved experimentally to hold for aqueous solutions over a range of wave-lengths from 10 to 200 metres. In each case, when $\frac{c_s}{c}$ becomes appreciable in magnitude, the effect is to increase the resistance that gives a maximum production of heat. The existence of this "capacity shift" is proved experimentally in a later part of this paper.

The conditions of practical radiothermy differ from those of the experiments in an important particular. In the latter we find the maximum heating by replacing a particular solution with its values of R and C, by solutions of different conductivity, whilst in the practical case, all the different substances are present simultaneously in the field, and we wish to know in which of them most heat is developed.

Suppose that we have a number or circuits like that of (c) arranged in parallel. Then for all of them the voltage V' is the same, and the power loss in each of them is given by the formula (6). Let us suppose that the nth circuit, say, consists of a volume of material, of cross section A, at right angles to the field, and length d, and that the total distance between its faces and the oscillator plates is d^1. Let its dielectric constant and conductivity be K and x

respectively. Then, see Fig. 4,

$$c = \frac{KA}{4\pi d}, \quad R = \frac{d}{Ax}, \quad c_s = \frac{A}{4\pi d^1}.$$

Substituting in (6) and putting $\omega = 2\pi\nu$, ν being the frequency, we find:—

$$P = \frac{(V')^2}{(d^1)^2}\,(A.d)\,\frac{\nu^2 x}{\nu^2\left(K+\frac{d}{d^1}\right)^2 + 4x^2}.$$

Or dividing by the volume, (Ad), the heat per unit volume produced per second

$$H = \frac{(V')^2}{(d^1)^2}\,\frac{\nu^2 x}{\nu^2\left(K+\frac{d}{d^1}\right)^2 + 4x^2}. \tag{11}$$

If $\frac{d}{d^1}$ is small compared to K, this becomes

$$H = \frac{(V')^2}{(d^1)^2}\,\frac{\nu^2 x}{\nu^2 K^2 + 4x^2}. \tag{12}$$

Let the circuits now be in series. In this case, the total current I' is the same for each of them, and the power loss in any one of them is given by (8). If the same condition, that $\frac{c_s}{c}$, which equals $\frac{d}{Kd^1}$, is small compared to unity, holds, this reduces to (10), *i.e.*,

$$P = (I')^2\left(\frac{c_s}{c_s+c_p}\right)^2\,\frac{R}{1+\omega^2R^2C^2}.$$

Substituting for R and C in terms of the dielectric constant, K, and the conductivity, x, we get,

$$P = (I')^2\,4\,\frac{d}{A}\left(\frac{c_s}{c_s+c_p}\right)^2\,\frac{x}{\nu^2K^2+4x^2},$$

and dividing by the volume, we find, for the heat per unit volume per second

$$H = 4\left(\frac{I'}{A}\right)^2\left(\frac{c_s}{c_p+c_s}\right)^2\,\frac{x}{\nu^2K^2+4x^2}. \tag{13}$$

Equations (12) and (13) show, on differentiation, that, even in the complex equivalent circuit of parallel and series combinations that represents the conditions of practice, the law of maximum heating is approximately (for constant K)

$$x = \frac{\nu K}{2}. \tag{14}$$

In fact, the heat produced per second per unit volume of any part of the body, where the dielectric constant is K and conductivity x, is given by

$$H = V^2 f\cdot\frac{\nu^2 x}{\nu^2K^2+4x^2}, \tag{15}$$

where f is a function determined by the shape, position, electrical properties of the portion considered and its neighbors, *but is not dependent upon the frequency.*

The condition that must hold for this to be approximately true is, as has been shown, that $\frac{c_s}{c} << 1$ or that $\frac{d}{d^1}$ is small compared to K. The dielectric constant of the majority of body substances is of the order of magnitude of that of water, *i.e.*, 80, for the frequencies employed in practice. We have an approximation to 10% then if $d^1 > \frac{d}{8}$, *i.e.*, if the distance of the body from the

plate of the oscillator is greater than ⅛th of the width of the body, *i.e.*, if it is greater than about one inch. In the usual conditions of medical practice this condition is satisfied. It must be noted, however, that in the case of diathermy with electrodes in contact with the body, the formula has no application, the problem being a much more complex one. In this case, if the various bodies are considered to be in series with each other, Equation (4) applies and there is a selection of the substance obeying relation (5); but if on the other hand, they are considered to be in parallel, Equation (3) shows that the best conductor is the most heated. Any actual case must be a complex combination of the two limiting cases. We can say, however, that increasing the frequency tends to favor in general the heating of the better conductor.

The Equation (15) for the heating can be deduced, quite simply if not rigorously, from the general equations of the electromagnetic field. The differential equation that must be satisfied at every point in the field is that deduced from Maxwell's equations for a conducting medium,

$$\frac{d^2R}{dt^2} + \frac{4\pi x}{K}\frac{dR}{dt} = \frac{1}{\mu K}\nabla^2 R, \tag{16}$$

together with the boundary conditions, that at every surface of separation of two media the quantity $\left(\frac{K}{4\pi}\frac{dR}{dt} + xR\right)$ is continuous along the normal to the surface. The condition that at the condenser plates the potential is sinusoidal then completes the description of the problem. In the solution of these equations for the case of the Hertzian oscillator, which closely resembles the present problem, it is shown that at distances small compared to the wave-length of the oscillator, the "induced", electromagnetic, contribution to the total electric field is negligible, and the field is approximately entirely "electrostatic". That such is the case for the field between the plates of a high frequency oscillator has been shown experimentally by Pariseau (8). This means, that within any region that is homogeneous, the distribution of the field, though dependent upon the properties of the medium there and upon its shape, etc., is independent of the frequency. (To say that "skin effect" is negligible is an equivalent statement.)

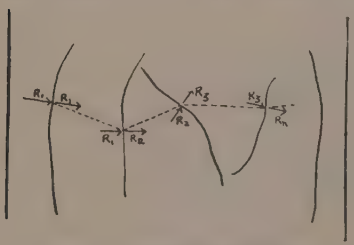


FIG. 5. *Electric field in body.*

The heterogeneous body may be considered to consist of a number of regions in which the properties are homogeneous, separated by "surfaces of separation". Within one of these regions we can find two points on the surface of separation where the normal component of the field, within the region, is the same. Let these points (as in Fig. 5) be joined by a line which is produced so that this is true for each region through which it passes. Then at the intersections of the line with the surfaces of separation of the regions, we have the equations for the normal induction:—

$$\frac{K_1}{4\pi}\frac{dR_1}{dt} + x_1R_1 = \frac{K_2}{4\pi}\frac{dR_2}{dt} + x_2R_2 = \ldots\ldots\ldots = \frac{1}{4\pi}\frac{dR_0}{dt}, \tag{17}$$

where R_0 is the normal intensity at the point of emergence of the line from the body to the air. Since the solutions for R_n must contain the time factor $e^{j\omega t}$, the equations become:—

$$R_1\left[\frac{K_1}{4\pi}\, j\omega + x_1\right] = R_2\left[\frac{K_2}{4\pi}\, j\omega + x_2\right] = \ldots\ldots\ldots\ldots = R_0\, \frac{j\omega}{4\pi}$$

or

$$R_n = R_0\, \frac{j\omega}{K_n j\omega + 4\pi x_n},$$

which gives, putting $\omega = 2\pi\nu$,

$$R_n^2 = R_0^2\, \frac{\nu^2}{\nu^2 K_n^2 + 4x_n^2}. \tag{18}$$

The average intensity $\overline{R_n}$ within the region (n) will be connected with the intensity R_n at the point of intersection of the line with the surface, by a relation that, as has been shown, does not involve the frequency ν. R_0 will be proportional to the potential difference between the plates of the oscillator, the factor of proportionality again being independent of the frequency. We have then the final equation

$$\overline{R_n}^2 = V^2 f\, \frac{\nu^2}{\nu^2 K_n^2 + 4x_n^2},$$

where f does not depend on ν. The heat produced per second per unit volume is, by a simple calculation, shown to be equal to $\overline{R_n^2} x$, and therefore we have,

$$H_n = V^2 f\, \frac{\nu^2 x_n}{\nu^2 K_n^2 + 4x_n^2},$$

which is identical with Equation (15).

In any practical case, the prediction of the absolute values of the rise in temperature of the various parts of a complex heterogeneous body is impossible, because of the complexity of the functions that involve the distribution of the electric field throughout the body. The formula does, however, give us very useful information. It indicates, that, if we wish to *favor* the heating of any particular region, we must choose the frequency ν of the oscillations so that the quantity $\frac{\nu^2 x}{\nu^2 K^2 + x^2}$ is greater for that region than for other regions.

Fig. 6 shows how this quantity varies with increasing frequency, assuming different values for the constants K and x. Curve 1 corresponds closely to that of blood,

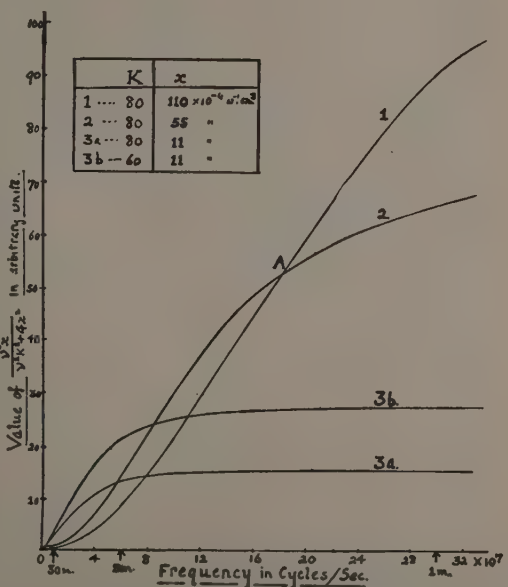


FIG. 6. *Graph showing change of relative heating of different substances with change of frequency.*

whilst 2 is for a substance of the same dielectric constant but having a conductivity of half the value. Curve 3*a* is for a substance of conductivity one-tenth that of blood, and corresponds to the curve for the proteins of the blood. The effect upon the curves of changing the value of K is seen by comparing 3*b* with 3*a*.

A practical application of such curves suggests itself as follows. Suppose that we wish to heat certain diseased cells in the body, without injury to the neighboring normal cells. Let the electrical constants of the normal cells be K, x, whilst those of the diseased cells are found to be K', x'. The curves with these parameters will intersect at some point such as A in Fig. 6. This may be described as a "point of inversion" for the two substances, for frequencies less than that of this point favor the heating of the one substance; frequencies greater than this favor the heating of the other. There is a very simple expression giving the frequency at which this inversion occurs. It is

$$\nu_c^2 = \frac{4xx'(x'-x)}{K^2x'-(K')^2x}, \tag{19}$$

which, in the special case of $K = K^1$ reduces to

$$\nu_c = \frac{2}{K}\sqrt{xx'}. \tag{20}$$

Frequencies less than ν_c will favor the heating of the substance for which the ratio $\frac{x}{K^2}$ is the lesser, frequencies greater than ν_c favor the heating of the other. (No point of inversion exists if $\frac{(x'-x)}{K^2x'-(K')^2x}$ is negative, in which case all frequencies favor the substance of the greater $\frac{x}{K^2}$.) From the values of the dielectric constants and conductivities of the diseased and of the normal cells we can thus deduce the frequency best suited to the end in view. More complete knowledge of the electrical properties of body substances for high frequencies would here appear to be desirable.

In the special case where the dielectric constant is assumed to be the same for all the substances, the favored substance is the one for which the relation, $x = \frac{K\nu}{2}$, is true. The conductivity in this equation is in absolute electrostatic units. A convenient practical form may be given to the relation which will apply to dilute aqueous solutions only. If λ be the wave-length of the oscillations in air expressed in metres, the conductivity of the solution that is most heated is

$$x = \frac{130}{\lambda} \times 10^{-4}\omega^{-1},$$

or if the frequency be N million cycles,

$$x = \frac{4}{9} N \times 10^{-4}\omega^{-1}.$$

From tables of conductivities the corresponding concentration can be found, which, for instance, would be the most suitable as a medium for biological preparations which it was desired to heat by the short waves.

Search is still being made, by workers in the biological and biochemical field,

for specific effects of the waves upon living cells other than those traceable directly to the heating effect. No definite evidence of such has yet been found. Apparent specific effects might be produced by a selective heating of particular cells greater than that which would be produced by a general heating of their surroundings by ordinary means. In the case of laboratory experiments, the foregoing equations provide a method of discrimination between the heating effect and any other effects of the waves that may be present. For by variation of the conductivity of the media used in the experiments, keeping other conditions the same, the amount of heat produced can be varied over a wide range, without seriously modifying any other specific effect of the waves.

Experimental Work

The shortest wave-length used in the previous experiments was 11.5 metres. In order to reach shorter wave-lengths a new oscillator had to be constructed and the technique of the measurements considerably altered. Its construction follows closely that described by Gill and Morrell (3, 4). The plate and grid inductances are simply two parallel brass tubes, supported horizontally about 10 cm. apart. They slide telescopically into other tubes, so that their total length can be adjusted from one to three metres. At one end they are connected to the grid and plate of a U.X.852 tube, and at the other they are bridged by a variable air condenser of high voltage insulation, of maximum capacity 50 microfarads. Here connections are made through air-spaced chokes to the d.-c. supply on the one side and to the grid-leak and earth on the other. With a plate voltage of about 1000 volts, and using a grid-leak of 25,000 ohms, the set will give oscillations of wave-length from 3 to 8 metres. The design is a convenient one, as by interchange of the usual voltages on the grid and plate the electron oscillations of Barkhausen and Kurtz are produced.

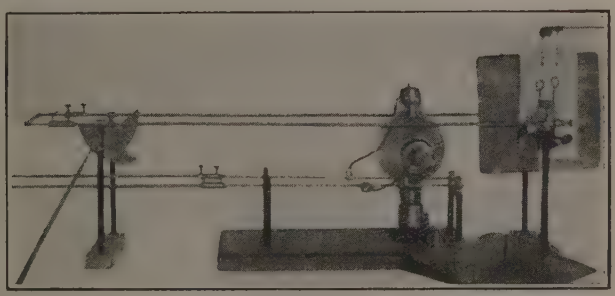


FIG. 7. *Arrangement of apparatus for experiments with 6.5-metre waves.*

With such high frequencies, it was quite impracticable to have the substance under test heated directly in the condenser of the oscillator, as its presence profoundly affected the output of the latter. A secondary circuit was therefore used, consisting of a variable frame of parallel rods, closed at one end and carrying the heater condenser plates at the other. This frame was supported just above the rods of the oscillator, and in each experiment, with the body to be heated in position, its length was adjusted until there was resonance with the oscillator beneath. Thus, although the body in the test condenser was changed, the frequency was kept the same throughout. The field in the

in fat. They are just visible in the photograph of the unpainted meat. In general, it was noted that the fat portions might be raised to a very high temperature—sufficient to cause 'frying'—while the lean portions had been heated comparatively very little. The fact that the specific heat of the fat is much less may partly explain this. The very white, outside, fat remained however comparatively cold. There is apparently a tendency for more heat to be developed at the 'corners' of the specimen due to the distribution of the electric field here.

The piece of beef in Fig. 10 shows similar characteristics of the heating—but here most heat was developed in the 'connective tissue' between two bones—(which latter seemed to heat least of all). Fig. 11 demonstrates clearly that the localization of heat is not determined solely by the concentration of electrical resistances and of specific heat in various parts, but is also due to a definite selective action of the waves. For here the same piece of meat (pork) has been heated both by 25-metre waves and by 10-metre waves and the course of the heating followed in each case. The change of color starts at one spot with the 25-metre wave and spreads to the fat portion to the right hand side in the photograph. Whilst with the 10-metre waves, the red color appears simultaneously in two places and subsequent spreading proceeds in a different manner. The condensers in which the experiments were made were geometrically similar, and to eliminate the effect of permanent changes in the meat produced by the heatings, these were made alternately, *i.e.*, first for a short period with 25 m., then for a short period with 10 m.; then for a longer time with 25 m., and finally for the longer time with 10 m. The progressive changes were repeated in each case, and there is no doubt that the change in wavelength had changed the distribution of the heating.

For use in further experiments, a thermocouple needle was constructed from a No. 22 gauge hypodermic needle, of length 2 in. An insulated No. 40 gauge constantan wire runs down the inside of the needle and forms a junction with the steel needle at the oblique orifice at its point, this orifice being closed with a touch of solder. The needle is mounted on a bakelite tube in which the cold junctions lie side by side, a steel wire of small diameter being brazed to the top of the needle so that the junction of dissimilar metals may be made inside the tube. 'Telephone' leads are the connections to the Cambridge potentiometer, which, with a Kipp and Zonen galvanometer, has a sensitivity sufficient to give readings of the temperature of the point of the needle quickly and accurately to $\frac{1}{50}$° C. The construction of such and of more elaborate thermocouple needles is described by Karrer and Estabrook (5). For more accurate work the cold junctions should be kept at constant temperature—in an ice bath—but for comparative measurements the needle described is more convenient.

With its aid, a study was made of the heating of a hen's egg by the short waves. The shape of an egg is such that the distribution of the electric field in it is more simple than for other shapes—approximating to a uniform field, if the interior were of homogeneous dielectric properties. Moreover, search is still being made by workers in this field for specific effects, other than those

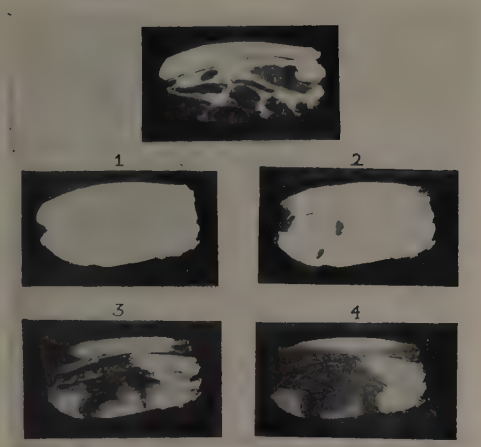


FIG. 9. *Heating of meat (pork) by 25-metre waves.*

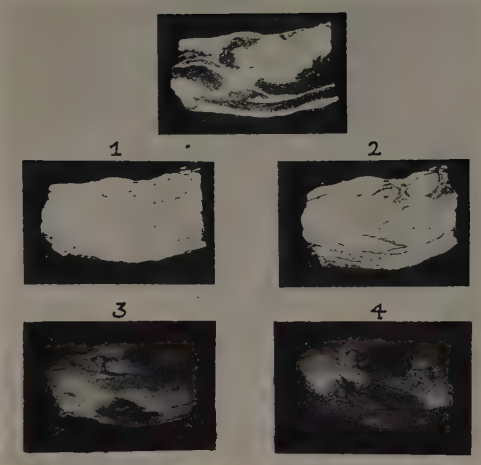


FIG. 10. *Heating of meat (beef) by 25-metre waves.*

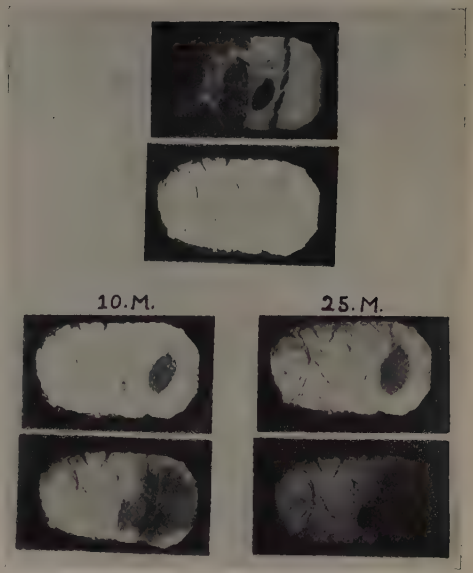


FIG. 11. *Heating of meat by 10-metre and by 25-metre waves.*

due to heating, of the short waves on living organisms, and attempts have been made to modify the development of the embryo in eggs. An enquiry into the physical process of heating of the interior of an egg might then be of service.

The eggs were hung in the field of the oscillator between the plates in a cradle of threads, with the long axis of the egg parallel to the electric field. The thermocouple needle was clamped vertically to an adjustable stand such that it could be racked up and down and the temperature at different points across a vertical diameter of the egg could be measured by insertion of the needle through a small hole in the shell. Initial measurements having shown that the interior was at uniform temperature, the egg was subjected to the oscillations for a given period. After heating, the egg was removed and the temperatures across the diameter again taken. If the interior of the egg were entirely homogeneous we should have a practically homogeneous electric field throughout, and would find that the resultant distribution was almost uniform, though cooling from the outside would make the temperature higher in the middle.

Results are shown graphically in Fig. 12. With the 25-metre waves there is distinct evidence that the temperature rise is greater in the white than in the yolk. The measurements must be given a qualitative significance only, as errors due to cooling, unequal conduction down the needle in the different positions and to other factors that are unavoidably present. The minimum temperature in the centre was, however, found repeatedly. When a wave-length of 10 metres was used, however, the eggs in no case showed this minimum, but in many cases a small increase in temperature at the centre, indicating that here the yolk heated as much if not more than the white. With the oscillator giving waves of 200 metres, there was evidence of the selective heating of the white as with 25 metres, but sufficient heat could not be produced with the equipment available to give reliable results.

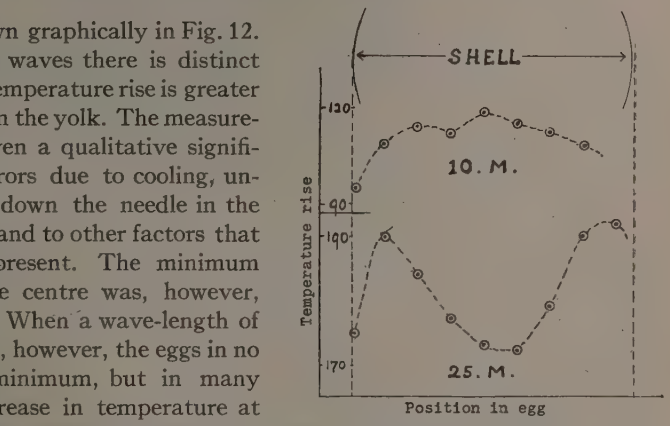


FIG. 12. *Rise in temperature of the interior of an egg heated by short waves.*

The white and yolk were then separated and heated together in the field under the same conditions. They were placed in two similar small test tubes hung side by side with their axes parallel to the field between the condenser plates. The rise in temperature produced by the oscillations was taken by a mercury-in-glass thermometer. Heating was very rapid indeed compared to that produced in the eggs indicating how great is the shielding effect of the shell. Results at the different frequencies are shown in Table I.

In the case of the 10-metre heating, the substances were interchanged in their test tubes and experiments repeated to eliminate effects due to unequal

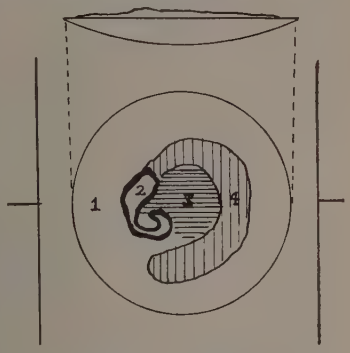
TABLE I

	10 Metres			25 Metres			190 Metres		
	Initial Temp., °C.	Final Temp., °C.	Difference, °C.	Initial Temp., °C.	Final Temp., °C.	Difference, °C.	Initial Temp., °C.	Final Temp., °C.	Difference, °C.
White	25.0	36.7	11.7	27.0	42.0	15	24.5	28.3	3.8
Yolk	25.5	43.5	18.0	27.0	48.0	21	25.0	29.0	4.0
Ratio: White / Yolk		0.65			0.70			1.05	

heating of the latter themselves. The difference amounted to 5%. For comparison double distilled water, and 0.002 *N* KCl solution were heated in the test tube by the 25-metre waves with the same time as above. The rises in temperature were 6° and 40° C. respectively. The conclusion is that under the same conditions of external field, shape and position, there is selective heating of the yolk at 25 metres; the ratio is increased at shorter wave-lengths; but at 200 metres the white heats more than the yolk. The specific heat of the yolk is less than that of the white, and this plays its part, but, of course, in the same way for each of the wave-lengths, so that by experiments at different frequencies we differentiate a true 'selective' effect from that due to specific heat differences. Yet in the egg we found greater heating of the white than of the yolk at 25 and at 200 metres, though the yolk has the advantage of lying nearer to the maximum for a given field. Thus the field induced in the white must be greater than that in the yolk. This is what we would predict from the difference in the dielectric constants of the two substances. Blüh (1) gives for a wave-length of 50 cm. the values—white 68.0, yolk 60.0. We have shown, in our previous paper, that whether the dielectric constant of the outer substance be greater or less than that of the inner, its effect is to shield the latter from the field; and if for our frequency the dielectric constant of the white be the greater, the concentration of lines of force would be the greater there. The heating of the egg is an excellent example of how the various factors, position, dielectric constant, conductivity, and wave-length play their parts.

To complete the investigation, some experiments were made with fertilized eggs. It was hoped that, by 'chandling', the position of the embryo could be seen, and after heating the egg in the field the temperature of the embryo could be taken with the thermocouple needle. This was not found practicable, so the shell was broken and the contents placed in a watch glass which was supported in the centre of the condenser field. After heating by the oscillations the temperature of the embryo and of the surrounding fluids could be found by the thermocouple. The diagram shows the relative positions of the contents

of the egg, and gives the relative rise in temperature of the parts after heating. Temperatures were taken in the order shown and then in the reverse order, to eliminate difference due to cooling. The measurements were repeated with an embryo that showed less development. The results in general are that the greatest rise in temperature is produced in the allantoic excretion — next of about equal amount in the area *opaca vasculosa* or yolk-sac, and in the body of the embryo, and least in the surrounding white. With 10 metres the relative heatings were about the same, with the relative heating of the body of the embryo increased.



Part	Rise in temp.
1. White	4.5°
2. Body	8.5°
3. Excretion	10.0°
4. Yolk-sac	9.5°

FIG. 13. *Rise in temperature of a fertilized egg heated by short waves.*

There is some evidence from experiments *in viva* on the heating of animals in the field of an oscillator of 30 metres wave-length, that the liver rises in temperature more than the heart (2). Experiments were made to find the relative rises in temperature of these media, *in vitro*, when under the same conditions in the field. The substances (calves' liver and heart) were packed into the two test tubes previously described and heated side by side in the field. Wave-lengths of 10, 25 and 200 metres were used, care being taken that the arrangement of the test tubes between the condenser plates was geometrically the same in the three cases. The results, given in the Table II, are very interesting.

TABLE II

	25 Metres				200 Metres				10 Metres			
	Initial Temp., °C.	Final Temp., °C.	Rise	Ratio	Initial Temp., °C.	Final Temp., °C.	Rise	Ratio	Initial Temp., °C.	Final Temp., °C.	Rise	Ratio
Liver	21	28	7.0	1.55	20	26.5	6.5	1.85	26.0	43.5	17.5	0.8
Heart	21	25.5	4.5	1	20	23.5	3.5	1	25.0	46.0	21.0	1

It will be seen that though at 25 metres the heating of the liver is favored, at shorter wave-lengths there is reversal. The results of experiments conducted *in vitro* cannot be transferred without caution to conditions *in viva*, but it may safely be concluded that if for any reason it were desired to enhance this selective heating of the liver in radiothermy, the longer wave-lengths would be more effective; and that at shorter wave-lengths the relative heating of the heart would become of more importance.

The results have, therefore, demonstrated the existence of the selective effect and its control by choice of wave-length. Whether or not the phenomena have any practical importance in the medical application, depends on whether the blood flow and rapid interchange of heat in the living body render the differences of temperature that the oscillations would undoubtedly produce, negligible or not. Experiments *in viva* upon animals will alone settle the point. There is already some evidence which has been referred to, in the heating of the heart and of the liver.

Summary

The theoretical basis of the formula previously given for the heat produced in a poorly conducting dielectric, which is placed in the field of a high frequency oscillator, has been examined in detail. Its application has been made to the complex practical case met with in medical radiothermy. Prediction of the selective heating effect is possible from a knowledge of the characteristic electric constants of the substances of the body, and by suitable choice of wave-length the heating of a particular part of a heterogeneous body may be favored over that of neighboring regions. The analysis applies with a satisfactory degree of approximation to the case of radiothermy, but not to electrode diathermy.

The previous experiments have been extended to shorter wave-lengths, and experiments on the heating of meat have demonstrated the selective action and its dependence upon wave-length. The longer wave-lengths favor the heating of the heart, shorter wave-lengths that of the liver. The rise in temperature of various parts of an egg has been measured as an example of the various factors that are involved.

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THE RANGE OF THE ALPHA-PARTICLES FROM URANIUM II[1]

By S. Bateson[2]

Abstract

The range of the α-particles from uranium II has been determined by a scintillation method to be 3.29 ± 0.08 cm. at 15° C. and 760 mm. This is in good agreement with Laurence's value found with a Wilson chamber. From the Geiger-Nuttall relationship the period is calculated to be 28,000 years, a value considerably less than that found recently by direct measurement.

Introduction

The range of uranium II was measured by Geiger and Nuttall (5) in 1912 using an ionization method. Their value of 2.9 cm. at 15° C. and 760 mm. was later corrected by Geiger (4) to 3.07 cm. In 1924 Gudden (6) calculated the range to be 2.91 cm. by measuring pleochroic haloes in fluorite. More recently Ziegert (15) has obtained the total ionization in air for several radioactive substances including uranium II. Calculation using the total ionization as given by Ziegert gives 3.13 cm. for the range of uranium II. Meanwhile Rutherford (11) had estimated that the range was probably not less than 3.23 cm. The most reliable determination of the range of uranium II was made by Laurence (7) who obtained a value of 3.28 ± 0.03 cm. using a Wilson chamber. In view of the wide variation in these values, it seemed advisable to check the range by a different method.

Method

The scintillation method was adopted as the most feasible. This method has been used successfully to determine ranges by Rothensteiner (10), Taylor (13), and more recently by Philipp (9).

The principal difficulty of the scintillation method lay in the low activity of uranium II. Its practicability depends on whether or not a sufficient number of particles can be counted to insure reasonable accuracy. The effect of using a thick layer of active material is to increase the straggling of the α-particles, making the range harder to determine as well as shortening its value. Since the source had to be thin its area was increased by placing it at the centre of curvature of a glass segment on which the active material was deposited. In this way variations in the distance from source to screen for various portions of the source were almost entirely eliminated.

A preliminary calculation was made of the total number of scintillations per minute to be expected from a layer of material having a thickness equivalent to 1 mm. of air. This value was approximately one scintillation per minute. It was decided that this number, although small, was sufficiently large to obtain good accuracy within a reasonable time.

[1] *Manuscript received September 5, 1931.*
Contribution from the Department of Physics, Dalhousie University, Halifax, N.S.

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Apparatus

The counting chamber consisted of a brass cylinder having a removable end plate for the introduction of the source. A hole in the opposite end was covered with a glass disk on which the zinc sulphide screens were waxed. The source was held in such a position that the screen was at its centre of curvature. The whole apparatus was connected to a manometer and pump, so that the pressure could be varied. Air was admitted through a drying tube. The temperature of the air enclosed was read by a thermometer sealed into the wall of the cylinder. A mechanical shutter could be interposed between the source and the screen.

The source was uranium oxide, U_3O_8. It was necessary to remove radium, ionium and polonium, all of which gave rise to α-particles. The rest of the radioactive elements either give rise to β-particles or have very short periods and are not re-formed once the parent radium is removed.

Ionium was removed by adding a small amount of its isotope, thorium, and precipitating with oxalic acid. Radium was next removed by adding barium chloride and precipitating with sulphuric acid. Polonium was eliminated by adding bismuth nitrate and precipitating with hydrogen sulphide. The uranium nitrate was then converted to U_3O_8 by ignition and ground as fine as possible in an agate mortar.

After several unsuccessful attempts to obtain a thin layer of U_3O_8 on the glass, a scheme was devised whereby the material was blown on by means of an air stream. The glass segment was previously coated with very soft wax. The air stream was allowed to pass over the material held in a glass tube, an electric tapper keeping the particles agitated. This scheme automatically sorted out the finest particles and deposited them in a thin layer on the surface of the glass. A source could be prepared very quickly and showed an even deposit under the microscope. The weight of uranium oxide deposited on the source was found to be 0.00045 gm. per cm^2 corresponding to 0.21 cm. of air approximately. The source had an area of 37.6 $cm.^2$ and was placed at a distance of 4.51 cm. from the screen.

The screens were prepared by smearing a cover glass with a very small quantity of castor oil, sifting a small amount of zinc sulphide (Glew's) on the oil and shaking off the excess. Several screens were tried. The one used in the final determination gave good scintillations at a distance of 3.15 cm. and at the same time had a very low natural effect of about one scintillation every 10 min.

The maximum number of α-particles to be expected within a circle of 0.29 cm. diameter, using the source described, was calculated. Then the area covered by the zinc sulphide was estimated by taking a micro-photograph of the screen, projecting it on squared paper and outlining a number of the crystals. When allowance was made for the area not covered with crystals, the efficiency of the zinc sulphide was 72%.

The screen was observed with a microscope (Watson "Holos" objective NA 0.45) giving a field 0.29 cm. in diameter. A weak light illuminated the

screen serving to keep the eye focussed. The natural effect of the screen was measured for each determination by closing the shutter. These "zero counts" were taken over approximately the same length of time as the direct counts. The mean temperature and the pressure were read and recorded with the aid of a dim lamp. The distance between the source and screen was calculated at 15° C. and 760 mm. in the usual way, all formulas being reduced by a set of nomograms, so that the pressure could be changed rapidly in the dark room.

Results

A series of counts was made at points ranging from 2.72 to 3.15 cm. equivalent distance from the source. This work was tedious, and required a total of about 60 hr. counting. The values were grouped, averaged and nine points obtained. The above procedure was essential to increase the accuracy of each point, since the probable error depends on the number of scintillations counted.

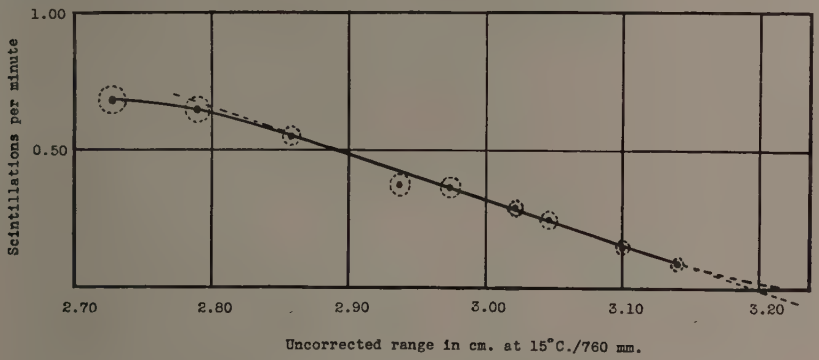

FIG. 1. *Uncorrected range of α-particles from uranium II.*

In the curve, Fig. 1, the ordinates represent the number of scintillations per minute, and the abscissas the distances from the source at 15° C. and 760 mm. The radius of each circle is proportional to the probable error.

The range was obtained in the usual way by drawing the tangent to the curve at the point of inflection, and producing it to intersect the abscissa. The point of intersection is seen to be at 3.20 cm. with an error of ± 0.02 cm. due to various possible slopes of the tangent.

Correction for Absorption by the Source

The fraction of the total number of tracks which are longer than $R+x$ is

$$z = \frac{1}{h\sqrt{\pi}} \int_x^\infty e^{-\frac{x^2}{h^2}} dx,$$

where h is a parameter determining the straggling of the curve. From this equation Laurence (7) has shown that a length of

$$\frac{1}{2}h\sqrt{\pi} + \frac{1}{2}m - m\left[2\left(\frac{2}{\sqrt{\pi}}\int_0^{\frac{m}{2h}} e^{-y^2}dy\right)\right]^{-1}$$

must be added to the range to correct for absorption by a source of finite thickness, where m is the thickness of the active layer in terms of cm. of air.

The distance from the range to the point of inflection is $\frac{h\sqrt{\pi}}{2}$. From this h was calculated for the particular curve. Substituting this together with the value of m for the source used, gives 0.09 cm. to be added to the intercept.

The error in measuring the radius of curvature of the source, in drawing the tangent and in reading the manometer amounts to less than ± 0.08 cm. equivalent distance in the range, giving the final range of the α-particles to be 3.29 ± 0.08 cm. at 15° C. and 760 mm.

This confirms Laurence's value of 3.28 ± 0.03 cm. for the range. It will be noted that the accuracy in his determination is considerably greater than that obtainable in this experiment.

Remarks

Before making the final determination a previous trial experiment had been performed, using a screen less sensitive toward the end of the range, and having a large natural effect. The range obtained was considerably shorter than the final one. The errors in the first case were large, but not great enough to explain the difference.

Curie (2) has concluded that ranges found by scintillation methods fall short of those found by the Wilson chamber. Her conclusions are based on a comparison of the scintillation curve for Ra F obtained by Rothensteiner (10) and her own curve for Ra F based on Wilson chamber data. The work of Destriau (3) on the scintillation curve for Ra F lends further support to Curie's theory.

Recently, however, Philipp (9) has obtained the range of Th C^1 by scintillations in good agreement with Meitner and Freitag's (8) value found with a Wilson chamber, the errors in his determination being quite low. Bearing in mind the types of screen used in the trial and final experiments on uranium II, it would seem, as Rutherford (12, p. 113) has recently suggested, that the earlier attempts exaggerated the straggling. The curve obtained by the scintillation method approaches that obtained with a Wilson chamber as the screen becomes more sensitive and the natural effect decreases; provided the optical system is good, so that feeble scintillations are not overlooked.

The Geiger-Nuttall Relationship

By applying the Geiger-Nuttall relationship to Laurence's value of 3.28 cm. for the range of uranium II, the transformation constant was calculated to be 7.9×10^{-13} sec^{-1}, giving a period of 28,000 years.

Since the error in his determination of the range is probably no greater

than ± 0.03 cm. it follows from the curve that the period is no greater than 43,000 years.

Recently the period of uranium II has been measured directly by Walling (14) and by Collie (1). Collie places a lower limit of 10^6 years for the period. Walling arrives at a value of 340,000 years with a calculated error of about 15%.

When Walling's value of 340,000 years and the range as given by Laurence are plotted, the resulting point shows a deviation of 4.0% in range from the Geiger-Nuttall curve, while the remainder of the uranium family show a mean deviation of only 0.8%. The difference here is greater than the experimental errors permit. This is shown in Fig. 2 where the circles indicate the errors in range. Uranium II is the only member of the family which is seriously out of line with the Geiger-Nuttall curve. For this reason it is desirable that further measurements of both range and period be made.

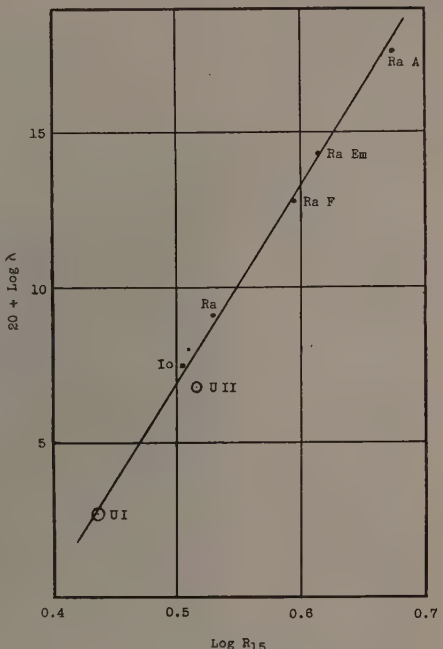

FIG. 2. *The Geiger-Nuttall relationship.*

Acknowledgment

The author wishes to thank Dr. G. H. Henderson for his encouragement and advice.

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RAMAN EFFECT OF BENZENE AND TOLUENE UNDER HIGH DISPERSION AND RESOLVING POWER[1]

By Leslie E. Howlett[2]

Abstract

This paper is a report of work carried out at the Macdonald Physical Laboratory on the Raman effect of benzene and toluene. The six-prism spectrograph, used by Dr. J. S. Foster for his work on the Stark effect, was employed in the investigation. It was found that a number of entirely new lines were observed in the Raman spectrum of both liquids. Some of these are due to the separation into components of previously observed lines; others are entirely new. Accurate measurements are given of the Raman frequency shifts and comparison with other results is afforded.

Introduction

Some previous work (1, 4, 5, 6) has been done on the examination of the Raman spectra of certain compounds under higher dispersion than is usually employed for this type of investigation. Hitherto the general result has been that the frequency differences characteristic of the molecules studied have been determined with greater accuracy but that no very new data have been revealed. It was felt by the writer that more work might be profitably done with the use of high dispersive and resolving power. The following discussion is the result of such investigations carried out on the much studied liquids benzene and toluene. The Raman spectra of these two substances have been very carefully studied, and the work has definitely shown that the use of spectrographs of high dispersive power is not only illuminating but quite necessary if the Raman spectra are to be thoroughly investigated.

Apparatus and Experimental Procedure

The spectrograph employed for this work was the six-prism one used by Dr. J. S. Foster of the Macdonald Physics Laboratory for his investigations on the Stark effect (2). Dr. Foster very kindly placed this instrument at the disposal of the author during the past summer. Since the details of its construction have been adequately described elsewhere (2), they are omitted in the present paper.

The success of Raman investigations depends to a large extent on the quality of the exciting source. The mercury arc serves as a very convenient and intense source of exciting radiation. It has the disadvantage, however, that after it has been in operation for some time the continuous spectrum increases very appreciably and as a result faint Raman lines are masked. Eventually even moderately strong ones are indistinguishable from the continuous background. Since it was desired to carry out this research under the most favorable conditions a new quartz burner was obtained. The lamp used was one of the Hanovia Research models. The wisdom of the preceding precaution was

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demonstrated by the fact that after about 150 hr. of operation the continuous spectrum had increased appreciably. At the end of 500 hr. it was strong enough to mask the fainter Raman lines.

The Wood method of irradiation was used in all experiments. Great care and special precautions had to be taken to have the whole set-up in perfect alignment, otherwise the loss of scattered light was so great that it was impossible to obtain a photograph of the Raman spectrum. Stray light had to be excluded with equal care.

It may be of interest to describe briefly the method of setting up the apparatus used by the author. A rigid stand to hold the scattering liquid and the associated lens system was constructed. A sketch of this is shown in Fig. 1. It consisted essentially of two pieces of brass plate fixed together by four brass rods. To one end was attached a piece of telescope tubing to carry the lens system for condensing the scattered light on the slit of the spectrograph. An extra sleeve, which fitted over this, could be extended right up to the slit of the spectrograph. In this way all stray light was cut out from this part of the path taken by the scattered light. The inside of both tubes was painted black. The Raman tube itself was of Pyrex and fitted snugly between the two brass plates. It was supported by two of the brass rods used to fasten these plates together. The Pyrex window just fitted into the entrance of the telescope tubing. The window was stopped down somewhat by painting a small black ring around its outer edge. This was effective in cutting out reflected light. The top of the Raman tube and the inside surfaces of the brass plates were covered with silver foil. Immediately below the Raman tube another similar tube rested on the other two brass rods securing the plates together. This, when filled with water or a filter solution, served as a lens for condensing in the scattering liquid the light of the mercury arc which was just below this last tube. A blast of air was sent between the plates for cooling purposes. This proved very effective.

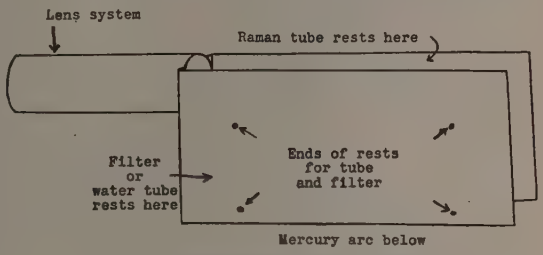

FIG. 1. *Diagrammatic sketch of apparatus.*

At the outset of the work this stand was set in perfect alignment with the collimator of the spectrograph by use of a small flashlight bulb set where the far end of the Raman tube would later come. Horizontal adjustment was then made to secure the proper relative positions for slit, lens and Raman tube. When this was done the stand was screwed rigidly in position. The line-up was checked at various times during the work but no further adjustments were required. The arrangement was found very convenient. It permitted change of the filter solution without disturbing the alignment or illumination in any way. If the arc went out during the exposure it could be started and put back in the same place with ease. In addition the iron arc could be photographed at the completion of the exposure by simply removing both tubes and

adjusting the position of the arc for focus on the slit. No change in the position of the lens took place. This last is an essential point to reduce as much as possible any chance of a shift in position of the iron lines on the plate with reference to the Raman spectra. Finally, it reduced the difficulty of alignment to a minimum. Great care was taken at the start and the work was finished. Thereafter only a check was required from time to time to make sure that all was still unchanged.

The plate-holder of the spectrograph is not curved and for this reason only a small section of the spectrum could be focussed on the plate at one time. On this account it was thought best to arrange for sharp focus of the region 4300-4700 Å. By so doing the higher frequency differences excited by 4046 Å and the lower ones excited by 4358 Å could be photographed on the same plate. The dispersion in this region ranged from 3-5.6 Å per mm. Ambiguity as to the origin of specific Raman lines was removed by the use of a quinine sulphate filter solution between the exciting source and the scattering medium during one of the exposures. Eastman-40 plates were found to be very suitable in the spectral region under consideration.

The iron arc was used as a comparison spectrum. It was superimposed on the Raman spectrum in the usual way. Measurements on the sharper and stronger Raman lines possess an accuracy of ± 0.3 cm^{-1}. In the case of the diffuse and faint lines the error should not exceed ± 1 cm^{-1}.

Merck's chemically pure benzene and toluene were used for the investigations. As a further precaution each liquid was freshly distilled before taking a Raman spectrogram.

Results and Discussion

Reproductions of the Raman spectrograms are shown in Fig. 2 and 3, respectively. The frequency differences are set forth in Tables I and II and are expressed in cm^{-1} in vacuum. Comparison is afforded between the results of the author and those of previous investigators. It will be noted that a fair number of extra frequencies are recorded by the writer which have not been previously reported. A number of these are, of course, due to the revelation of complexity under high dispersion of lines hitherto observed as simple. Others such as 807.2 and 842.4 cm^{-1} in toluene cannot be attributed to such an origin.

A glance at the reproduction of the Raman spectrograms and the tables of frequency shifts reveals how valuable is the use of high dispersion and resolving power. The principal advantages can be summed up under three headings:

1. Increase in the accuracy of the measurements.
2. The individual characteristics of the lines, *i.e.*, diffuseness, sharpness and gradation of intensity are more clearly shown.
3. A number of Raman lines are shown to be complex in structure, whereas smaller dispersion and resolving power had previously indicated them as simple. This may apply even to lines which are comparatively sharp under moderate and low dispersion.

Against these advantages is the objection to long exposures, which are

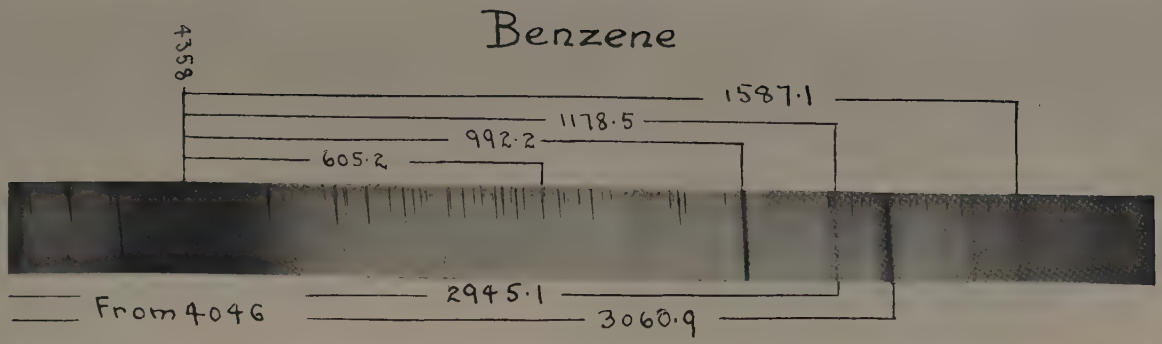

Fig. 2

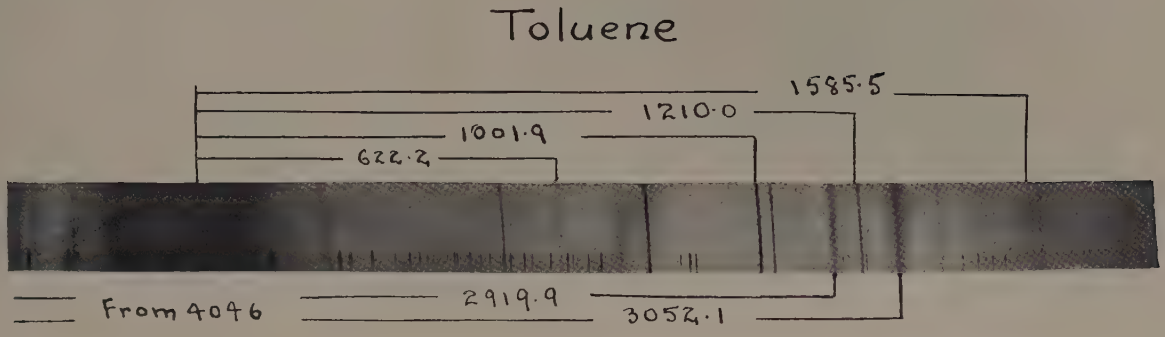

Fig. 3

FIG. 2 AND 3. *Raman spectrograms of benzene and toluene.*

TABLE I

COMPARISON OF FREQUENCY DIFFERENCES FOR BENZENE OBTAINED BY VARIOUS INVESTIGATORS

Dabadghao (1)	Langer and Meggers (4)	Soderqvist (5)	Howlett	Dabadghao (1)	Langer and Meggers (4)	Soderqvist (5)	Howlett
3184.0 (2)	3185.0	3184.8 (2)	3186.1 (1)	—	—	—	1005.3 (0)
3160.5 (0)	—	3162.9 (1)	3163.1 (0)	—	—	—	998.8 (5)
3062.3 (4)	3060.1	3061.3 (4)	3060.9 (8)	991.2 (8)	992.1	991.3 (5)	992.2 (10)
3048.5 (1)	3045.4	3046.9 (1)	3047.1 (5)	—	—	—	983.9 (5)
2950.8 (2)	2946.9	2946.8 (2)	2945.1 (2)	—	—	—	980.3 (5)
1607.4 (1)	1604.5	1604.1 (1)	1604.9 (4)	848.7 (10)	848.6	849.1 (0)	849.4 (4)
1583.4 (1)	1585.2	1583.6 (1)	1587.1 (4)	603.9	605.7	604.6 (2)	605.2 (5)
1178.1 (1)	1177.3	1179.0 (1)	1178.5 (5)	—	—	—	—

NOTE:—*Exciting wave numbers: 24704.78, 22938.09 cm*$^{-1}$.

Numbers in parentheses are the visual estimates of intensities on a basis of 10 for the strongest Raman lines.

TABLE II

COMPARISON OF FREQUENCY DIFFERENCES FOR TOLUENE OBTAINED BY VARIOUS INVESTIGATORS

Langer and Meggers (4)	Soderqvist (5)	Howlett	Langer and Meggers (4)	Soderqvist (5)	Howlett	Langer and Meggers (4)	Soderqvist (5)	Howlett
—	—	3205.9 (0)	1604.5	1603.2 (1)	1604.7 (3)	—	—	968.3 (0)
3053.9	3053.7 (5)	3062.5 (4)	—	—	1585.5 (2)	—	—	842.4 (0)
—	—	3052.1 (5)	1379.4	1377.3 (1)	1379.5 (2)	—	—	807.2 (0)
—	—	3035.4 (3)	1208.7	1208.6 (3)	1210.0 (5)	785.8	785.6 (4)	786.7 (8)
—	—	3004.4 (0)	—	1154.1 (1)	1180.3 (3)	622.6	621.2 (0)	622.2 (3)
—	2981.2 (1)	2982.3 (0)	—	—	1156.5 (3)	521.0	519.3 (2)	519.8 (3)
2920.2	2919.6 (2)	2919.9 (4)	1031.3	1027.6 (2)	1027.7 (6)	—	332.6 (0)	335.0 (0)
—	—	2865.8 (0)	1003.3	1001.6 (5)	1001.9 (10)	217.3	217.5 (1)	216.7 (4)
—	—	1628.5 (00)	—	—	992.1 (3)	—	—	—

TABLE III

COMPARISON OF FREQUENCIES OF BENZENE AND TOLUENE

Benzene	Toluene	Benzene	Toluene	Benzene	Toluene	Benzene	Toluene
3186.1 (1)	3205.9 (0)		1628.5 (00)	1005.3 (0)	1027.7 (6)	604.6 (5)	622.2 (3)
3163.1 (0)	3062.5 (4)	1604.9 (4)	1604.7 (3)	998.8 (5)	1001.9 (10)		519.8 (5)
3060.9 (8)	3052.1 (5)	1587.1 (4)	1585.5 (2)	992.2 (10)	992.2 (3)		
3047.1 (5)	3035.4 (3)			983.9 (5)			335.0 (0)
	3004.4 (0)		1379.5 (2)	980.3 (5)	968.3 (0)		216.7 (4)
	2982.3 (0)		1210.0 (5)				
2945.1 (2)	2919.9 (4)	1178.5 (5)	1180.3 (3)	849.4 (4)	842.4 (0)		
	2865.8 (0)		1156.5 (3)		807.2 (0)		
					786.7 (8)		

necessitated by powerful spectrographs and the almost excessive care which is necessary in the alignment of the whole experimental arrangement to prevent undue loss of light. The first is not a really serious objection. If the spectrograph is set up on a good solid base in a place where temperature variations

are not wide, and a further precaution is taken to have a thermostatic device in the prism chamber, the greater part of the objection to long exposures is removed. If a mercury arc is used as the source of incident radiation one can operate this with very little attention for a long period of time. To set up the outfit for an exposure requires considerably more care and attention, but the improved results, it is felt, are well worth this added difficulty.

It is not in general possible to obtain anywhere near the precision of which one's instrument is capable when measuring Raman lines. The general diffuseness and faintness of many lines renders it impossible to approach the real precision of the instrument. It is felt, in this connection, that some have claimed greater accuracy for their results under low dispersion than was justified. The variation of the measurements on the same substances by different writers bears out this statement. Even with the large dispersion and resolving power employed in these experiments it was found that the average spread of the measurements made on the same line at different times but with equal care amounted on the average to about 1.1 cm^{-1}. In some cases it amounted to as much as a 2 cm^{-1}, in others not more than 0.6 cm^{-1}.

The high dispersion and resolving power shows with great clarity the variation in the individual characteristics of the lines, *i.e.*, sharpness, diffuseness, gradation, etc. Some lines have the sharpness of the incident exciting radiation, others have a width which runs to several Ångstroms or more. In the case of the latter, where there is no question of two Raman lines, coming from different exciting lines, being superimposed, there seems to be no departture from symmetry, *i.e.*, there seems to be no shading off either to the red or to the violet. This diffuseness is due, it is suggested, to an unresolved rotational structure superimposed on a pure vibration. The fact that some lines are quite sharp (these are in the minority) and others are diffuse or very diffuse seems to imply that partial rotations are possible. It is intended to study further the present plates for possible band systems.

In benzene all Raman lines except the group at 1,000 cm^{-1} are more or less diffuse. Those in the neighborhood of 3,000 cm^{-1} show the characteristic most markedly. The frequency 3047.1 is the most diffuse of these and its edges are poorly defined. The very strong frequency 3060.9, although broad has more sharply defined edges. The lines representing frequencies 1604.9 and 1587.1, characteristic of the double bonded carbon atom in the ring structure, are narrower than 3060.9 and yet can still be termed quite broad. Frequencies 604.6 and 849.4 are of about the same order of diffuseness; 1178.5 is somewhat sharper. The group near 1,000 cm^{-1} stands out unique in sharpness. These lines are of the same order of sharpness as the incident radiation. Two of them, 980.3 and 983.9, approach the sharpness of iron lines.

In toluene the lines representing the hydrogen frequencies are also broad and diffuse. The frequency 2865.8 is especially broad extending over 8-10 Å. The line 3052.1 is sharply defined, broad and quite intense. It resembles very strongly in appearance 3060.9 found in benzene. The other hydrogen lines are all diffuse but of varying degree. Of the remaining lines 216.7 is extremely broad and diffuse, while 786.7, 992.2, 1001.9, 1027.7 are comparable for

sharpness with the incident radiation; the others are moderately broad with 519.8 and 1210.0 standing out as the sharpest.

There is no indication of asymmetry in any of the Raman lines characteristic of benzene and toluene.

A glance at Table III in which the frequencies of benzene and toluene are arranged side by side shows how analogous are the two Raman spectra. The spectrum of toluene is the richer in Raman lines but the general grouping is the same. There is even a further similarity. A number of lines can be selected from each spectrum which correspond closely not only in frequency but in appearance. In Table III this point is illustrated by the underlining of such frequencies in each spectrum. It has been noted before that certain frequencies, very slightly modified, appear in the spectra of aromatic compounds. It appears even under high dispersion that, at least in the case of benzene and toluene, these lines have not only similar frequencies but have also the same appearance.

The groups of lines representing the hydrogen vibrations of both benzene and toluene have been shown in Fig. 4 and an approximate curve drawn to indicate the distribution of intensity. The similarity is rather striking as one would expect.

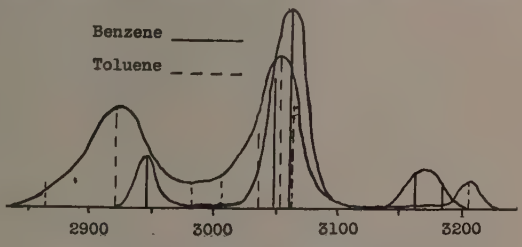

FIG. 4. *Visual estimates of the intensity distribution in the hydrogen vibrations of benzene and toluene.*

The group of lines near 1,000 is perhaps the most interesting. It is a strong line in this region which has hitherto been considered so characteristic of an aromatic compound. It is suggested that this frequency is closely linked with the symmetric vibration of the ring formation. It has always been noted by previous investigators as strong and sharp. The high dispersion and resolving power employed in these experiments brings out the fact that although less powerful instruments reveal it as sharp and simple it is really complex. In benzene it consists of five components, which are all quite sharp, of which some are comparable to the iron arc in sharpness. In toluene this group has three and possibly four components. There is some doubt as to whether 968.3 cm. should be included or not. Visually its appearance is quite unlike the others. It is quite broad. In proximity it is very close to the rest and for this reason should perhaps be included with them. Further investigations on other aromatic compounds would settle this point and provide interesting data on the modifications of this series as the hydrogen atoms of the benzene nucleus are substituted by various groups.

Such complexity, as is revealed in this case alone, indicates quite clearly, as must have been evident before, that postulated mechanical models of atoms bound by springs cannot possibly provide the basis for an accurate discussion of the Raman effect, however interesting and in many cases helpful these

models may be. As has been indicated by others and the present writer (3) these methods may be used with the aid of classical mechanics to predict the approximate order of Raman frequencies. The problem of dealing with Raman frequencies theoretically must be left to quantum mechanics for full and accurate solution. The complexity of the group at 1,000 cm^{-1} emphasizes this point. Such frequencies could not be deduced from the simple spring models but until the quantum mechanics is developed to the point where it can deal with such complicated molecules, the latter will, of course, serve a useful purpose. The gathering of careful and exact data on the Raman effect under high dispersive and resolving power will be important in that it will show of just what a complete theory has to take care. The fine points will not be revealed by low powered instruments although, of course, much important work has and will continue to be done by such instruments.

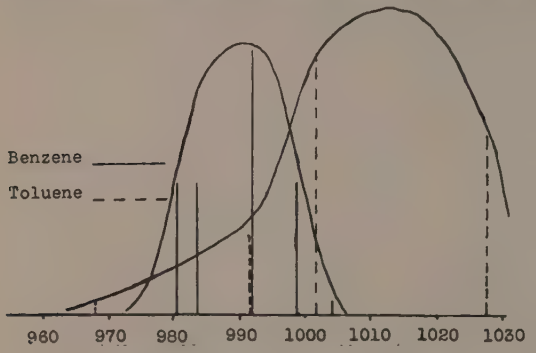

FIG. 5. *Visual estimates of the intensity distribution of the Raman group near 1000 cm^{-1} in benzene and toluene.*

Fig. 5 has been included to indicate diagrammatically the distribution and intensity of the lines of the group near 1,000 cm^{-1} in both benzene and toluene. It is shown that the intensity centre is shifted towards the higher frequencies in toluene.

The two lines of benzene representing 1604.9 and 1587.1 cm^{-1}, which originate in the vibrations of the double bonded carbon atom, seem to be replaced in toluene by three lines of the same order of frequency. Two of these, 1628.5 and 1585.5, have not been hitherto observed. The former line is very faint when due to excitation by 4358 and is masked by the nebulosity surrounding 4358, near which it appears, when excited by 4046. There seems, however, no question that it is a true frequency shift from 4358 as observed on the writer's plates.

The two lines 1180.3 and 1156.5 seem to take the place of the one 1154.1 observed by Söderqvist. It is interesting to note that Langer and Meggers did not observe any line at all in this region. On the present plates these two lines appeared quite as strong as the group near 1600 when a quinine sulphate filter was used. They are also quite strong when excited by 4046. This is not the first time that one author has observed fairly strong frequencies which another has failed to observe at all: acetone is another example. Such discrepancies are worthy of note. The writer suggests they may, to some measure, originate in the continuous spectrum which becomes so pronounced in the mercury arc after some use. He observed that on plates taken towards the end of the best period in the life of the mercury arc, the lines 1180.3 and 1156.5 and others in this region were becoming masked much more rapidly than those near 1600.

The justification of the assignment of the lines representing shifts 807.2, 842.4, 968.3, 992.1, 1585.5, 1628.5, 2865.8, 3004.4, 3035.4, 3062.5 and 3205.9 cm^{-1} rests on the use of a quinine sulphate solution as a filter between the arc and the scattering medium. The experimental data were not considered sufficient until at least two good spectrograms of each liquid had been obtained. The first one employed the full radiation of the mercury arc; the other used a quinine filter.

Acknowledgment

The author wishes to express his gratitude to Dr. A. S. Eve, Director of the Macdonald Physics Laboratory, McGill University, whose interest in this work made arrangements for its execution possible.

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STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES

XXXIX. STRUCTURE OF THE CELLULOSE SYNTHESIZED BY THE ACTION OF *ACETOBACTER XYLINUS* ON GLUCOSE[1]

By Harold Hibbert[2] and Jacob Barsha[3]

Abstract

A description is given of the properties of the cellulose obtained from glucose by the action of *Acetobacter xylinus*.

Acetylation of the product gives a yield of 98.8% of a triacetate identical with cellulose triacetate, and the cellulose regenerated from the acetate is identical with the starting material. The triacetate, when spun dry from solution in chloroform, gives a silk-like fibre which on de-acetylation yields a fibre showing the same X-ray diffraction pattern as natural cellulose. Acetolysis of the acetate yields cellobiose octacetate.

Treatment of the triacetate with methyl alcohol containing HCl gives a yield of 94.1% of α- and β-methylglucosides, while on direct hydrolysis of the cellulose with a solution of zinc chloride in hydrochloric acid, a practically quantitative yield (99.5%) of glucose is obtained.

Simultaneous de-acetylation and methylation of a partially saponified acetate soluble in acetone gave trimethyl cellulose (yield, 84.6%). The latter, on hydrolysis with methyl alcohol containing HCl, yielded 2:3:6-trimethyl methyl-glucoside (yield, 92.3%) which, in turn, was converted into crystalline 2:3:6-trimethyl glucose (yield, 83.5%). The last two compounds were found to be identical in every way with the same products prepared from ordinary cotton cellulose. It follows from this that the cellulose obtained by direct bacterial synthesis from glucose is identical with natural cellulose.

Introduction

The constitution of cellulose, in so far as the purely chemical side of the question is concerned, seems now to be definitely established (9, 10). Hydrolysis of cellulose results in a quantitative yield of glucose (13, 17) and it is interesting to note that in the present investigation, for the first time, it has been possible to demonstrate the reverse reaction, namely, the polymerization of glucose to cellulose, by the action of bacteria, the simplest form of plant life. However, the mechanism by which cellulose is formed in the plant is still unknown and it would seem that some information regarding this mechanism might be obtained by studying the products of the action of bacteria on simple carbohydrates and related products.

The selection of the bacterial polymerization product from glucose as the first substance to be investigated seemed to be a natural choice. This substance has been shown, as indicated in the experimental part, to be identical

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Since completion of this work an article has appeared by E. Schmidt, M. Atterer and H. Schnegg (Cellulosechemie 12: 235-241. 1931) dealing with the chemical properties of a synthetic cellulose obtained from sucrose by bacterial action. The authors, however, have restricted their investigation to X-ray analysis, hydrolysis and acetolysis.

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[3] *Holder of bursary under the National Research Council of Canada, 1930-31.*

with natural cellulose. A large number of other carbohydrates and related compounds such as fructose, sucrose, mannitol and glycerol, have been submitted to the action of *Acetobacter xylinus* and the chemical constitution of the resulting membrane is being actively investigated.

A. J. Brown, one of the earliest investigators in this field, during his studies on *Bacterium aceti* (2, 3, 4), or as he sometimes called it "vinegar plant", found that the organism formed extremely tough membranes when cultivated in suitable nutrient solutions containing carbohydrates. The membranes thus formed yielded a reducing sugar upon hydrolysis with sulphuric acid and were readily soluble in a solution of cuprammonium hydroxide. These properties, and the results of combustion analysis, led him to believe that the membrane was cellulose. In his report (3) he stated, "This production of cellulose by a simple cell plant, and its use as a cell connecting medium, seems of great interest in view of the important part which cellulose plays in a similar manner in the more highly organized forms of the vegetable kingdom; and it appeared that any information that could be gained, as to the materials from which cellulose is formed by the "vinegar plant" might perhaps assist in better understanding of the complex reactions which take place in higher plants".

Brown was able to obtain a similar type of membrane from fructose, mannitol and glucose. He found that the one prepared from fructose yielded a dextrorotatory sugar upon hydrolysis with sulphuric acid.

Emmerling (7) obtained membranes by using Bertrand's Sorbose bacterium (1), the latter identical with Brown's *Bacterium xylinum*. These products were only slightly soluble in cuprammonium hydroxide, and contained between 2 and 3% of nitrogen. On treatment of the pellicle with hydrochloric acid a crystalline product was obtained which resembled glucosamine hydrochloride, and which led him to conclude that the original membrane did not consist entirely of pure cellulose, but that a chitin-like substance was also present.

C. A. Browne (5) found that a cellulose-like fermentation of sugar cane juice was of fairly frequent occurrence in Louisiana, and he described the causal organism as being probably identical with Brown's *Bacterium xylinum*. He investigated the cellulose membrane from sugar cane juice but was unable to confirm Emmerling's results.

Eggert and Luft (6) studied membranes prepared by E. Schmidt, who apparently obtained them by the action of *Acetobacter xylinus* on sucrose, and from the dried preparations obtained X-ray diagrams similar to those from β-cellulose. Ipatjef (14) measured the rotation in a solution of cuprammonium hydroxide of presumably similar material and obtained a value quite similar to that for cellulose.

In no instance has a detailed study of the synthetic formation of cellulose from glucose by bacterial action been made, nor has there been any thorough chemical study made of the polymerized product. The purpose of the present investigation was, first, to examine the method of formation of the cellulosic material by the action of *Acetobacter xylinus*, and, secondly, to carry out an exhaustive chemical investigation of the products obtained from various carbohydrates and related products by the action of this organism with a view

to throwing more light on the true nature of plant synthesis.

Discussion of Results

The "cellulose" whose constitution is the subject of this investigation was prepared by the action of *Acetobacter xylinus* on glucose according to the method outlined in a previous communication (21).

It was possible, by washing, to obtain the "cellulose" free from reducing sugars and from nitrogen compounds. This latter fact stands in contrast to the observation of Emmerling (7) who obtained membranes containing between 2 and 3% of nitrogen. He assumed the latter was due to the presence of a chitin-like material in the membrane, but it now seems more probable that the nitrogen found was part of the nutrient material originally employed.

The air-dried membranes obtained in the present research still contained between 5 and 7% of moisture and about 0.3% of ash which could not be removed by boiling with water. Estimation of carbon and hydrogen on a sample dried at 100° C./15 mm. for 10 hr. gave values establishing the empirical formula $(C_6H_{10}O_5)_x$.

Owing to the compactness of the dried membrane, it proved to be somewhat more inert than natural cellulose to chemical reagents. The acetylation reaction was found to be the best means for bringing the membrane into a form such as would facilitate the determination of its chemical constitution. Acetylation with a mixture of acetic acid and acetic anhydride, using sulphuryl chloride as catalyst, produced an almost quantitative yield of a triacetate having the same properties as the triacetate prepared from cotton cellulose. A similar acetylation was also carried out using concentrated sulphuric acid as catalyst.

De-acetylation of "cellulose" triacetate by saponification with 2*N* alcoholic sodium hydroxide yielded a regenerated "cellulose" which, because of its powdery nature, proved to be more amenable to the action of hydrolytic agents.

Partial de-acetylation of the triacetate produced an acetate containing about 40% acetyl (CH_3CO-) which was completely soluble in acetone and similar in this respect to the commercial acetates used for the manufacture of artificial silk.

Dry-spinning of a chloroform solution of the triacetate yielded a silk-like fibre which on complete de-acetylation by immersion in 2 *N* alcoholic sodium hydroxide solution yielded a fibre of regenerated cellulose. X-ray examination of the latter proved its identity with natural cellulose.

Hydrolysis of the triacetate with methyl alcohol containing 0.75% HCl gave a 94% yield of a mixture of α- and β-methylglucosides. Direct hydrolysis of the "cellulose" with a hydrochloric acid solution of zinc chloride showed an almost quantitative conversion of the "cellulose" to glucose, thus proving the former to be composed entirely of glucose building-units.

A simultaneous de-acetylation and methylation of the acetone-soluble acetate gave a yield of 84% of trimethyl cellulose, the latter on hydrolysis with HCl and methyl alcohol yielding the characteristic 2:3:6-trimethyl

methylglucoside (yield 92.3%). Further hydrolysis with 5% HCl gave crystalline 2:3:6 trimethyl glucose (yield 83.5%).

The above data, a synopsis of which is given in the accompanying table, prove conclusively the identity of the synthetic cellulose in question with normal cotton cellulose.

TABLE I

COMPARISON OF THE PROPERTIES OF COTTON CELLULOSE WITH SYNTHETIC CELLULOSE OBTAINED BY THE ACTION OF *Acetobacter xylinus* ON GLUCOSE

Cotton cellulose				
Cellulose triacetate (15)	α- and β-Methyl glucoside obtained from cellulose triacetate (15)	Trimethyl cellulose (11)	2:3:6-Trimethyl methylglucoside (16)	2:3:6: Trymethyl glucose (16)
$\alpha_D = -22.3°$ (chloroform) (c = 0.8092) Yield: 99.5%	$\alpha_D = +106\text{-}108°$ (equilibrium rotation in methyl alcohol—HCl) (c = 0.970) Yield: 95.5%	$\alpha_D^{16} = -10.0°$ (chloroform) (c = 1.04) Yield: 85-98% M.p. = 215-216°C. $OCH_3 = 45.6\%$	$\alpha_D^{20} = +66.0°$ (chloroform) (c = 1.34) Yield: 95% B.p. = 115-118° C./0.5 mm. n = 1.4590	$\alpha_D = +66.5°$ (methyl alcohol—HCl) (c = 1.35) Yield: 86% M.p. = 94-104°C.
Synthetic cellulose				
$\alpha_D^{22} = -21.9°$ (chloroform) (c = 1.0140) Yield: 98.8%	$\alpha_D^{22} = +107.5°$ (equilibrium rotation in methyl alcohol—HCl) (c = 0.9996) Yield: 94.1%	$\alpha_D^{22} = -9.2°$ (chloroform) (c = 2.226) Yield = 84.6% M.p. 231-232°C. $OCH_3 = 44.0\%$	$\alpha_D^{23} = +64.4°$ (chloroform) (c = 2.495) Yield: 92.3% B.p. = 111.5-115° C./0.35-0.40 mm. n = 1.4560 (25°C.)	$\alpha_D = +65.2°$ (methyl alcohol–HCl) (c = 1.14) Yield: 83.5% M.p. = 95-97° C.

The synthetic cellulose is soluble in cuprammonium hydroxide, acidified zinc chloride solution and yields the usual viscose solution on treatment with carbon bisulphide and alkali.

Experimental

In collaboration with H. L. A. Tarr (21), the "cellulose" was prepared from glucose by the action of *Acetobacter xylinus*. It is to be noted that in the preparation of the membranes, a nutrient medium was used which did not itself produce growth with the bacteria so that the membrane may be regarded as being produced entirely from the carbohydrate employed. Control experiments were carried out in connection with each batch of material.

The product was made up of an infinite number of closely compacted membranes. The whole was enormously swollen so that a membrane, which in the swollen condition weighed about 200 gm., yielded only 2 gm. of dry substance, indicating that it had taken up about 10,000% of its own weight of water.

Purification was effected by boiling with repeated changes of distilled water

for about one week, at the end of which time the wash waters no longer gave a reducing action when tested with Fehling's solution. The washing process was interrupted at intervals and the membranes were centrifuged in a basket centrifuge so as to expel most of the water. It was possible to obtain, by this method, a product which was not only free from reducing sugars but one which was also free from nitrogen as shown by the usual sodium fusion test on the dried material.

The "cellulose" so obtained from glucose exhibits most of the properties of natural cellulose. It is completely soluble in Schweitzer's reagent, in zinc chloride solution, and is insoluble in all ordinary organic solvents. The dried membrane looks very much like parchment paper and shows no fibrous structure when examined under the microscope. It has great tensile strength. The product showed even greater resistance than cellulose to the action of the various chemical reagents employed in the determination of its structure.

When dried in air, the "cellulose" retains between 5 and 6% of moisture but this can be removed on heating for several hours, at 110°C./15 mm. in the presence of phosphorus pentoxide. Analysis:— Found: C, 44.79; H, 6.23%: calcd. for $(C_6H_{10}O_5)_x$; C,44.42; H, 6.2%.

Preparation of the Triacetate

Method (*a*). *Using sulphuryl chloride as catalyst* (*15*). Five grams of "cellulose" (moisture, 6.55%) was left in contact for 44 hr. at 18-20°C. with 37.5 cc. of glacial acetic acid. Then 37.5 cc. of glacial acetic acid, through which a dry stream of chlorine gas had been bubbled for 45 sec., was added to the mixture. After standing for half an hour, 90 cc. of acetic anhydride was added and SO_2 gas bubbled through the mixture for 60 sec. It was then stirred rapidly for one hour at room temperature. The reaction flask, which was fitted with a mercury-seal stirrer and reflux condenser, was immersed in a water bath at 65° C. and kept at this temperature with rapid stirring for three hours. The mixture was allowed to stand overnight at room temperature. The viscous syrup was diluted with 200 cc. of chloroform and a large excess of water (4 litres) added. The chloroform was then evaporated whilst the mixture was being stirred continuously. During this process, the acetate separated in granules which were washed with water until free from acetic acid, and then dried at 60° C./10 mm. To remove traces of free acetic acid, the product was extracted with ether in a Soxhlet extractor. Yield, 8.20 gm. (98.8% of theory).

The acetate was completely soluble in chloroform and in acetylene tetrachloride, somewhat soluble in pyridine and only partially soluble in acetone. It showed in chloroform solution $[\alpha]_D^{22} = -21.9°$ (c=1.0140).

The acetyl content of the acetate was determined by the method of Murray, Staud and Gray (18). The "cellulose" acetate was weighed out using a 0.5-gm. sample and transferred to a 250-cc. Erlenmeyer flask. To this was added 20 cc. of pyridine. The flask was loosely stoppered and warmed at 53° C. with occasional shaking until the acetate had completely dissolved. This required about 10 min. To the flask was now added 20 cc. of 0.5 *N* sodium hydroxide solution while shaking gently to disintegrate any precipitate

formed. The flask was then tightly stoppered and placed in a bath at 53° C. for 30 min. At the end of this time, the sides of the flask were washed with 25 cc. of distilled water, two drops of phenolphthalein were added and the excess of alkali titrated with standard sulphuric acid until the solution just became colorless. Analysis:—Found: $COCH_3$, 44.4: calcd. for $(C_{12}H_{16}O_8)_x$; $COCH_3 = 44.8\%$.

Method (b). Using concentrated sulphuric acid as catalyst. Dry "cellulose" (10 gm.) was added at room temperature to a mixture of 37.5 cc. of glacial acetic acid, 37.5 cc. of acetic anhydride and 1 cc. of concentrated sulphuric acid in a glass centrifuge jar. Heat developed at the start and the jar was cooled in a beaker of cold water. The reaction mixture was allowed to stand at room temperature for half an hour and then stirred rapidly for $4\frac{1}{2}$ hr. The viscous, golden-yellow syrup thus obtained was diluted with 150 cc. of glacial acetic acid and then centrifuged (4,000 r.p.m.). The solution, which was poured off from a small amount of undissolved material, was not quite clear and was clarified, after further dilution with 150 cc. of acetic acid, in the following manner: A Büchner funnel, carrying a very fine filter paper, was covered with a fairly thick layer of fuller's earth which had been deposited from an acetic acid suspension. The solution was poured on this and on applying suction came through perfectly clear. As the rate of filtration decreased, the surface of the fuller's earth was scraped lightly with a spatula and the rate of filtration thereby much increased.

The acetate was precipitated by allowing the clear solution to drop into 600 cc. of distilled water with rapid stirring. The precipitated product separated in a swollen condition and was separated by centrifuging. The acetate was again suspended in water, centrifuged and the process repeated using alcohol instead of water. The acetate, which still contained a great deal of absorbed liquid, was dried in the vacuum oven (60° C./15 mm.) in the presence of solid NaOH. It was dissolved in 17.5 cc. of chloroform and this solution placed in a 2-litre three-necked flask fitted with a mercury-seal stirrer and a condenser for downward distillation. A litre of distilled water was then added, and the mixture heated and stirred rapidly, thus removing the chloroform. The acetate was obtained in the form of snow-white granules which were filtered off and washed with water until the filtrate gave no precipitate with barium chloride. After drying, the acetate was extracted with ether in a Soxhlet extractor for five hours to remove free acetic acid. Yield, 16.2 gm. (91.5% of theory). $\alpha_D^{22} = -21.3°$ in chloroform ($c = 0.6930$).

Analysis: The material was dried at 78° C./15 mm. over P_2O_5. Found: $COCH_3$, 44.6; calcd.: $COCH_3$, 44.8%.

Regeneration of "Cellulose" from the Acetate

The triacetate (2 gm.) was covered with 50 cc. of 2*N* alcoholic sodium hydroxide solution and allowed to stand for 24 hr. The mixture was filtered and washed thoroughly with distilled water. The regenerated "cellulose" was suspended in water and a few drops of dilute HCl added to neutralize any adsorbed alkali. The mixture was again filtered and washed thoroughly until

no precipitate was given with silver nitrate solution. The product had the same fibrous appearance as the acetate from which it had been prepared. It was snow-white in color, non-reducing to Fehling's solution and was somewhat more reactive towards reagents than the original dried membrane. Its physical properties were identical with those of the original "cellulose" pellicle. Yield, 1.16 gm. (81.9% of theory).

Preparation of acetone-soluble "cellulose" acetate (19)

The triacetate (5 gm.) was heated with 60 cc. of 95% acetic acid and 2 gm. of sodium acetate for about 36 hr. at 98° C. This treatment produced a partial saponification of the acetate rendering it soluble in acetone. The soluble acetate was then precipitated from the solution in the same way as in the case of the triacetate.

*Spinning of Triacetate and X-ray Examination**

A 15% solution of the triacetate in chloroform was spun "dry", using the apparatus developed in the Kaiser-Wilhelm Institut für Faserstoffchemie, Berlin-Dahlem (8), and a silk-like fibre obtained. Complete saponification of this fibre by immersion in 2 *N* sodium hydroxide solution for 24 hr., followed by thorough washing, yielded a regenerated "cellulose" fibre. The latter on X-ray examination was found to be identical with fibres of natural cellulose.

Hydrolysis of "cellulose" triacetate with methyl alcohol. Formation of α- and β-methyl glucosides (15)

The triacetate (5 gm.) was heated with 75 cc. of methyl alcohol containing 1% of HCl in a sealed bomb-tube at 125° C. for 50 hr. At the end of this period, only a trace of solid remained undissolved and the liquid had assumed a golden-yellow color. The slight amount of acid remaining after this treatment was neutralized with silver carbonate, and the filtrate was decolorized with charcoal. The clear liquor was evaporated to a syrup under diminished pressure in a tared flask. The solvents were completely removed by evaporation at 60° C./10 mm. in a current of dry air to constant weight. It was found that crystallization could be accelerated by dissolving the resulting syrup in a small amount of hot alcohol and allowing the solution to stand for a few hours. The alcohol was then evaporated at 60-70° C. under reduced pressure. The material in the flask was completely crystalline and almost white. Yield, 3.17 gm. (94.1% of theory).

The crystals of α- and β-methylglucosides melted at 105-130° C. In a solution of methyl alcohol containing 1% HCl, the mixed glucosides showed a rotation of $\alpha_D^{22} = +95.2°$ (c = 0.9996). After heating in a sealed tube at 100° C. for three hours, a portion of this same solution increased in rotation to +107.5°.

Fractional crystallization of the mixed glucosides from absolute ethyl alcohol yielded the characteristic crystals of α-methylglucoside melting at 164.5° C. In water $[\alpha]_D^{20} = +157.2$ (c = 1.01). The mother liquor from the recrystallization now contained a large excess of the β-methylglucoside. Evaporation and heating in a bomb-tube for five hours at 125° C. with methyl alcohol con-

*The X-ray data are to form the subject of a separate communication.

taining HCl resulted in a re-establishment of the equilibrium and a constant rotation of +107.3° was found. This last result indicates that no compounds other than α- and β-methylglucosides were present.

Hydrolysis of "Cellulose" with Zinc Chloride-hydrochloric Acid

The method employed was that used by Hibbert and Percival (13) in the hydrolysis of cellulose. The hydrolytic agent was a solution of anhydrous zinc chloride (1 part) in two parts of concentrated hydrochloric acid (d. 1.180) and was of $d_4^{22} = 1.454$. The material used was regenerated "cellulose" prepared in the manner described above. This was used in preference to the "cellulose" itself because the former dissolved more rapidly in the solvent employed. About 0.25 gm. of the material, dried at 60° C./15 mm., was accurately weighed into a dry, glass-stoppered bottle and 50 cc. of the solvent added by means of a pipette. The mixture was shaken vigorously for about 20 min. at the end of which time solution had occurred. To obviate a slight cloudiness which obscured the reading of the polarimeter, the solution was filtered through a tared Gooch crucible with minimum suction to avoid loss of hydrogen chloride. The Gooch crucible was washed with distilled water and alcohol, dried at 60°C./20 mm., weighed and the increase in weight subtracted from the original weight of "cellulose" employed.

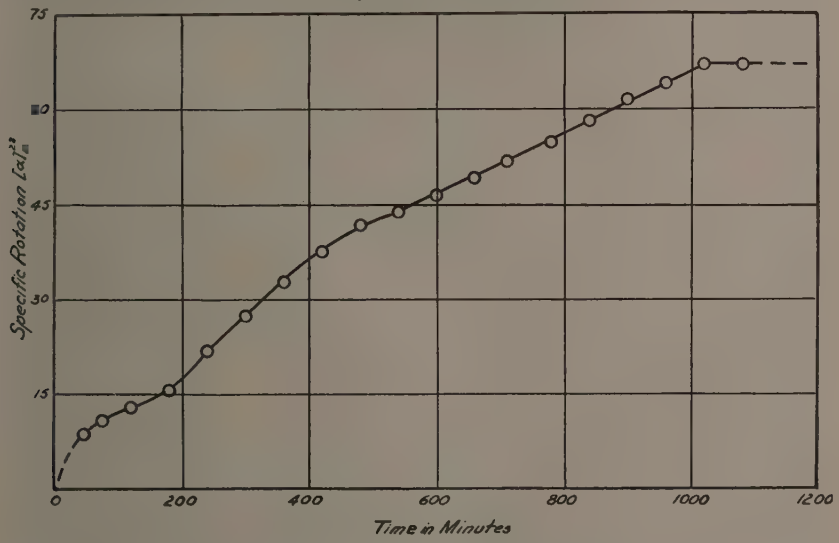

FIG. 1. *Hydrolysis of "cellulose" in zinc chloride-hydrochloride acid solution.*

The clear solution was transferred to a jacketed polarimeter tube kept at 23° C. ± 0.2° C., and readings were taken at intervals until the rotation became constant. $[\alpha]_D^{23}$ was calculated on the basis of glucose, *i.e.*, $[\alpha]_D^{23}$ observed $\times 162/180$. The rotation of pure anhydrous glucose in the same solvent gave $[\alpha]_D^{23} = +67.3°$ (c = 1.0028). The results are given in Table II and plotted in Fig. 1.

TABLE II
EXPERIMENTAL RESULTS

Time, in min.	α_D^{23}	Time, in min.	α_D^{23}	Time, in min.	α_D^{23}	Time, in min.	α_D^{23}
0	0	300	27.3	660	49.2	1020	67.0
45	+ 8.9	360	32.8	720	51.9	1080	67.0
75	10.9	420	37.6	780	54.7	1140	+67.0
120	13.0	480	41.7	840	58.1		
180	15.7	540	43.7	900	61.5		
240	21.9	600	46.5	960	64.2		

NOTE:—*Concentration—0.4566 gm. of cellulose in 100 cc. of solvent.*

The above figures indicate quite clearly that the "cellulose" is hydrolyzed completely to glucose.

Preparation of Trimethyl "Cellulose"

Direct methylation of the dried membrane was attempted in a preliminary experiment. This was carried out according to the usual method of Haworth in which the membrane, cut up into small pieces, was treated with dimethyl sulphate and 30% NaOH solution. The reaction was carried out in a three-necked flask fitted with a stirrer and the reagents added from separate dropping funnels. At the end of the reaction, the starting material was unchanged in physical appearance. Analysis showed it to contain only 10.4% methoxyl. It appeared as if the same difficulty would be encountered here, as in the case of cotton cellulose, which as shown by Hess (12) requires ten methylations before the theoretical number of methyl groups can be introduced.

Recourse was then had to a simultaneous de-acetylation and methylation treatment of an acetone-soluble "cellulose" acetate according to the method which was applied recently by Haworth, Hirst and Thomas (11) to ordinary cellulose acetate. Dry acetone-soluble "cellulose" acetate (12.00 gm.; CH_3CO = 39.4%) was dissolved in 240 cc. of acetone and treated with 385 cc. of 30% NaOH and 145 cc. of dimethyl sulphate in 10 equal portions at intervals of 15 min. The reaction was carried out at 56° C. in a one-litre three-necked flask fitted with a reflux condenser and a dropping funnel for the dimethyl sulphate. The NaOH solution was added from a dropping funnel through the condenser whilst the third neck was fitted with a mercury-seal stirrer. After the second addition, a gelatinous precipitate formed on the sides of the flask. This was returned to the reaction mixture by scraping with a glass rod and by vigorous stirring. The emulsion, which formed during subsequent additions, thickened towards the end of the reaction. Care was taken to maintain an alkaline reaction throughout the methylation. The acetone was then distilled off on the water bath, the mixture heated to 85° C. and filtered through a steam-jacketed Büchner funnel. The solid was washed with one litre of boiling distilled water. The light-brown granular product was dried, ground to a powder and triturated with 300 cc. of boiling water. After being heated for several hours on the steam bath, it was filtered again and washed with one

litre of boiling water. Yield, 8.44 gm. (92.2% of theory calc. for trimethyl cellulose). CH_3O, 40.7%; calcd. for $(C_9H_{16}O_5)_x$; CH_3O, 45.6%.

The product was then subjected to a second methylation using the same procedure as before. The reagents were added in 10 equal portions at intervals of 30 min. Yield, 8.20 gm. Analysis: Found: CH_3O, 42.2%.

This material was subjected to further methylation by means of Purdie's reagents. The reaction was carried out in a three-necked flask fitted with a spiral reflux condenser, the upper end of which was closed with a calcium chloride tube, a mercury-seal stirrer to keep the silver oxide in suspension, whilst the third neck, serving for the introduction of the pure, dry silver oxide, was closed by a cork.

The methylated product was dissolved in 100 gm. of methyl iodide in the flask, which was then immersed in a bath at 46-47° C. A clear solution was obtained. Silver oxide (40 gm., dried at 50° C./15 mm.) was added in four-gram portions every 30 min. At the beginning of the eighth addition, 25 gm. of methyl iodide was added to the reaction mixture which had become quite thick. After the last addition, the flask was heated for a further hour at 50° C. with vigorous stirring. Chloroform (approximately 100 cc.) was now added and the mixture stirred for 15 min. The silver salts were separated by centrifuging and then extracted four times with boiling chloroform (150 cc. each time) under reflux. The united chloroform extracts were dried over anhydrous magnesium sulphate, filtered and evaporated to a volume of 150 cc. The methylated product was obtained as a snow-white precipitate by dropping the chloroform solution, previously cooled to 0° C. into 700 cc. of cooled dry ligroin (b.p. 30-50° C.) while stirring rapidly at 0° C. It was filtered off, washed with more ligroin and dried over sulphuric acid in vacuum. It was dried further at 78° C./15 mm. over P_2O_5. Yield, 8.06 gm. Analysis: Found: CH_3O 43.6%.

A second methylation was carried out with Purdie's reagents exactly as described above. Yield, 7.75 gm. (84.6% of theory). The snow-white powder was soluble in chloroform and benzene; it dissolved slowly in ice-water but was insoluble in boiling water. Analysis:—Found: wt. 0.02185 gm. gave 0.07277 gm. AgI; CH_3O, 44.0; calcd. for $(C_9H_{16}O_5)_x$; CH_3O, 45.6%. In chloroform $[\alpha]_D^{22} = -9.2°$ (c = 2.226); in benzene $[\alpha]_D^{22} = -14.3°$ (c = 1.706). M.p., 231-232°C. For methylated cellulose with slightly lower methoxyl content than the theoretical, Haworth, Hirst and Thomas (11) also found a higher melting point and numerically smaller rotations than they found for completely methylated cellulose.

Hydrolysis of Trimethyl "Cellulose"; Preparation of 2:3:6-Trimethyl Methylglucoside (16)

Trimethyl "cellulose" (1.9 gm.) and 30 cc. of absolute methyl alcohol, containing 1% of HCl, were heated in a sealed tube at 100° C. for 95 hr. The solution was almost colorless and only a slight amount of flocculent material remained undissolved. The solution was neutralized with silver carbonate, filtered and decolorized with charcoal (Darco). The solution was evaporated

in a tared flask under diminished pressure and dried to constant weight at 100° C./15 mm. A colorless, viscous liquid remained in the flask. Yield, 2.03 gm. (92.3% of theory calculated for the conversion of trimethyl cellulose to trimethyl methylglucoside). The syrup was distilled at low pressure. A colorless, viscous syrup (1.50 gm.) was obtained. B.p. 111.5-115°C./0.35-0.40 mm. Refractive index, 1.4560 (at 25° C.). In chloroform $[\alpha]_D^{23} = +64.4°$ (c = 2.495). Analysis:—Found: wt. 0.02779 gm. gave 0.10933 gm. AgI; CH_3O, 52.0%; calcd. for $C_{10}H_{20}O_6$; CH_3O, 52.6%.

Preparation of 2:3:6-Trimethyl Glucose (12, 16).

Trimethyl methylglucoside (1.4 gm.) and 75 cc. of 5% hydrochloric acid were heated on the water bath under reflux for 11 hr. The solution was neutralized with barium carbonate, decolorized with charcoal and evaporated to dryness under reduced pressure. The residue was extracted with ether, dried with anhydrous magnesium sulphate and filtered. The ether solution was then concentrated to a volume of 25 cc. On cooling to −10° C., a portion of the 2:3:6-trimethyl glucose crystallized out and was filtered off. The mother liquor was evaporated under reduced pressure. The resulting syrup crystallized after seeding with a crystal from the first crop. Yield, 1.1 gm. (83.5% of theory); m.p. 95-97° C. The specific rotation in methyl alcohol became constant after catalysis with a trace of HCl. $[\alpha]_D^{24} = +65.2°$ (c. = 1.14). M. p. after recrystallization from dry ether, 105-108°C. Irvine and Hirst (16) found m.p., 104-108°C. Analysis:—Found: H, 8.36; C, 48.78%: calcd. for $C_9H_{18}O_6$; H, 8.2; C, 48.63%.

"Cellulose" Acetolysis

The procedure followed was that described by Spencer (20). One preliminary experiment was carried out, in which two grams of "cellulose" was treated with a mixture of 8 cc. of acetic anhydride and 0.2 cc. of concentrated sulphuric acid at 50° C. for 14 days. Treatment of the reaction product as outlined by Spencer yielded 0.75 gm. of crude cellobiose octacetate. After one recrystallization, a snow-white crystalline octacetate was obtained, m.p., 221-221.5° C. In chloroform $[\alpha]_D^{22} = +39.0°$ (c = 1.424).

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A SYNTHESIS OF THE METHYLTRYPTAMINES AND SOME DERIVATIVES[1]

BY RICHARD H. F. MANSKE[2]

Abstract

Because of the interest attached to N-methyltryptamine on account of its occurrence as an integral part of the calycanthine molecule, the free base and some of its derivatives have been synthesized. An account of the synthesis of N,N-dimethyltryptamine together with some carbolines derived from 1-methyltryptamine is also included. Finally, a detailed procedure for an improved preparation of tryptamine is given.

N-methyltryptamine has become of some interest because of its occurrence as an integral part of the alkaloid calycanthine. In a recent communication the author (5) recorded the degradation of calycanthine to benzoyl-N-methyltryptamine, but the free base was not obtained at that time and its preparation, together with some of its derivatives, is now placed on record.

Considerably more difficulty was encountered in the synthesis of N,N-dimethyltryptamine but sufficient was ultimately obtained for characterization. When tryptamine is treated with methyl iodide, all four possible products are obtained. The removal of the quaternary iodide is comparatively simple because of its sparing solubility in water or alcohol and the greater portion of the unchanged tryptamine can be separated from the mixture of bases on account of its sparing solubility in ether. Benzoylation of the residual mixture left the tertiary base intact and it was extracted from the crude product by means of dilute acid. The accumulated impurities were then removed by conversion to the picrate and the latter was recrystallized until pure. The picrate thus obtained consists of pale yellow needles sparingly soluble in water and melting at 168°C*. In melting point and composition this picrate closely resembles that of a base ($C_{12}H_{16}N_2$) isolated from *Withania somnifera* by Power and Salway (6, p. 496) but the question of identity was settled in the negative by the preparation of the free base which has the unexpectedly low melting point of 47°C. Furthermore, the hydrochloride could not be obtained crystalline although the pure base was used in its preparation. Aside from analysis the constitution of the base was proved by conversion to the previously described (5) quaternary iodide.

Another route which also led to the tertiary amine was via the quarternary chloride (from the iodide) by distillation *in vacuo*. The yield in this case as well as in the previous case was rather unsatisfactory and purification through the picrate was essential.

The physical properties of this series of bases are of some interest since the cases where all three representatives (primary, secondary and tertiary) have been obtained crystalline are extremely rare. Aside from a general effect in

**All melting points are corrected.*

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raising the melting point the indol nucleus is sufficiently removed from the nitrogen atom so that the effect of the successive introduction of methyl groups in lowering the melting point is probably not ascribable to any other cause. The melting points are: primary 117°, secondary 90°, tertiary 47°C. Parallel with the decrease in melting point a marked increase in solubility in organic solvents is observed. While tryptamine is soluble only with difficulty in boiling ether, the tertiary base dissolved rapidly in this solvent at 0°C.

In one attempt to methylate tryptamine with methyl iodide in acetone there was obtained, in addition to the quarternary iodide, a base or mixture of bases which yielded on benzoylation a beautifully crystalline product giving analytical figures in good agreement with $C_{20}H_{20}ON_2$. The substance gives a greenish blue color with Ehrlich's reagent, a fact which is indicative of substituents at positions 2 and 3 in the indol nucleus and probably also of ring closure. These properties and its mode of formation point to the substance being 2:2-dimethyl-3-benzoyl-2:3:4:5-tetrahydro-3-carboline, the gem-dimethyl group and the 2-carbon atom obviously originating in the acetone used as solvent. The mechanism of the synthesis recalls the usual isoquinoline synthesis, its facile occurrence being due to the great reactivity (3) of the 2-position in the indol nucleus.

Späth and Lederer (7) have described the synthesis of a number of carbolines from 1-methyltryptamine. The benzoyl derivative recently described by the author (5) has now been converted into the corresponding dihydro-carboline. Oxidation with chromic acid yields the carboline which exhibits the usual bluish fluorescence to a marked degree. Incidentally it may be pointed out that the phthalimido derivative of 1-methyltryptamine is admirably suited to the purification of the crude base owing to its ease of formation and sparing solubility even in boiling alcohol.

In view of the comparatively large amounts of tryptamine required for this investigation considerable attention was devoted to effecting improvements in yields at various stages, the final result being that the utility of the Ewin's (2) synthesis has been greatly enhanced.

The final condensation in the synthesis still leaves something to be desired in the matter of yields, but it must be kept in mind that in reality three reactions,—hydrolysis of the acetal, formation of the phenylhydrazone, and ring closure of the latter,—are carried out in one operation. The most serious obstacle heretofore has been the preparation of diethyl γ-aminobutyracetal from the difficultly accessible β-cyanoacetal (8). It has now been found that the comparatively simple preparation of the β-bromoacetal from acrolein has obviated much experimental difficulty in preparing the cyanide. The conversion of the bromide into the cyanide proceeds smoothly in boiling methanol, the troublesome autoclave conditions and the use of glycerol as solvent, essential in the case of chloroacetal, being entirely avoided. Furthermore, the yields are much better, and if the details given in the experimental section are observed the aminoacetal may be obtained in 85% yield from the cyanide.

The preparation of tryptamine is again detailed and finally attention may be

directed to an error in the recorded melting point (given as 138°C.) of benzoyltryptamine. The correct value (174°C.) is that first recorded by Asahina and Mayeda (1) and the incorrect value (due to Ewins) was erroneously introduced in writing the manuscript. It may also be pointed out that Ewin's melting point for tryptamine (145-146°C.) could never be confirmed. If there are in reality two crystal forms the higher melting form must of necessity be the most stable. Nevertheless, the distilled base which crystallized while still hot, that recrystallized from chloroform, that from alcohol, and the resolidified melt, all melted at 118°C. Asahina records 120°C., while Majima and Hoshino (4 p. 2045) give 114.5-115.5°C. On the other hand the melting points of the picrate (242-243° C.) and the hydrochloride (246°C.) given by Ewins are substantially within experimental error.

Experimental

N-Methyltryptamine

A rapidly cooled solution of 32 gm. of tryptamine in 300 cc. of chloroform was treated with an equal weight of methyl iodide and the mixture kept in cold water. When the first appreciable evolution of heat had subsided and a considerable amount of colorless syrupy salt had separated, the mixture was placed in an ice chest and allowed to remain for 3 days, during which time the salt largely crystallized. The solvent was decanted off and the residue dissolved in 120 cc. of ethyl alcohol. On cooling, a copious yield of the quaternary iodide was obtained. This was recrystallized once and it then melted at 197°C.

The combined mother liquor was freed of alcohol, dissolved in water, basified and the mixture extracted with chloroform. A further small amount of the quaternary iodide was obtained at this stage. It was recrystallized from alcohol (m p. 197°C.) and together with the first amount weighed 12.1 gm. The chloroform extract was clarified with sodium sulphate, the solvent distilled, and the residue distilled *in vacuo*. While still warm the distillate was treated with a small volume of chloroform and as crystallization proceeded ether was added. The mixture was then thoroughly cooled and the tryptamine filtered off and washed with ether; recovery, 15.1 gm.

It is possible to isolate N-methyltryptamine from the mother liquor of the tryptamine by fractional precipitation with petroleum ether, but the procedure is so tedious and the yields so low that a description is felt to be of no value. The mixture of primary, secondary, and tertiary bases which weighed 9.3 gm. in the above experiment was benzoylated as already described and the chloroform solution extracted with dilute hydrochloric acid (*A*).

Recovery and purification of the benzoyl derivative and boiling under reflux for 24 hr. with an excess of alcoholic potassium hydroxide (methyl alcoholic potassium hydroxide is inadequate as a hydrolytic agent, presumably because of the lower temperature of boiling) yielded the crude base. It was isolated from the alcohol-free hydrolysate by extraction with ether and removal therefrom with dilute hydrochloric acid. Regeneration of the base, extraction and distillation *in vacuo* yielded a colorless viscous distillate which was dissolved

in a little chloroform and cautiously treated with petroleum ether. Crystallization was rapid. After thorough chilling the base was filtered off, washed first with a little chloroform-petroleum ether and then with the latter solvent. N-methyltryptamine as thus obtained consists of stellate aggregates of needles, several isolated crystals showing rectangular form; m.p., 90°C.; yield, 4 gm. Analysis: Calcd. for $C_{11}H_{14}N_2$; C, 75.86; H, 8.05; N, 16.09%. Found: C, 76.07; H, 8.17; N, 15.90%.

The hydrochloride was obtained in colorless elongated plates with pyramidal terminations from alcohol-acetone or alcohol-ether; m.p., 180°C. Analysis: Calcd. for $C_{11}H_{15}N_2Cl$; Cl, 16.90%. Found: Cl, 16.96%.

It may finally be observed that hydrolysis of benzoyl N-methyltryptamine obtained from calycanthine yielded the base and hydrochloride identical with the above.

The picrate was obtained when an alcoholic solution of the base was treated with picric acid in the same solvent. Benzene was added and the solution evaporated to a small volume and the concentrate treated with much ether. The picrate then crystallized out in large plates closely resembling azobenzene in color. After thorough washing with ether it melted at 191°C. It is very sparingly soluble in hot water.

The phenylcarbamyl derivative of N-methyltryptamine was readily obtained by heating the base in chloroform with a slight excess of phenylisocyanate and evaporating to a small volume. Addition of ether caused the derivative in question to crystallize in large elongated hexagonal plates, which when recrystallized from methanol-ether melt sharply at 153°C. With Ehrlich's reagent it gives an immediate red color with a slight orange cast. Analysis: Calcd. for $C_{18}H_{19}ON_3$; N, 14.33%. Found: N, 14.47%.

N, N-Dimethyltryptamine Picrate

The acid extract (*A*) from the benzoylation of the tryptamines was basified and extracted with ether. Removal of the solvent and distillation of the residue yielded a pale yellow viscous oil which failed to crystallize under a variety of conditions; yield, 1.0 gm. It was therefore converted into the picrate which was twice recrystallized from alcohol, washed with ether, and then recrystallized from hot water in which it is sparingly soluble; m.p., 168°C. Analysis: Calcd. for $C_{18}H_{19}O_7N_5$; C, 51.80; H, 4.56; N, 16.78%. Found: C, 51.95; H, 4.75; N, 16.62, 16.53%.

N,N-Dimethyltryptamine

A hot aqueous solution of trimethyl-β-3-indolyl-ethylammonium iodide was treated with a 50% excess of freshly precipitated silver chloride and the mixture gently boiled for 15 min., during which time the larger lumps were frequently disintegrated with a stout glass rod. The silver halide was filtered off and the clear filtrate rapidly evaporated, preferably in a stream of air. The addition of a small amount of methanol and the cautious treatment of the solution with acetone yielded a copious crop of crystals of the quaternary chloride. The yield of crystalline product is well in excess of 80% if the procedure is rapidly carried out. Recrystallization by solution in a small

volume of methanol and cautious addition of acetone yielded colorless many-sided stout crystals melting at 193°C. The chloride in contrast to the iodide is extremely soluble in water and alcohol, but only sparingly so in acetone. Analysis: Calcd. for $C_{13}H_{19}N_2Cl$; Cl, 14.87%. Found: Cl, 14.06%..

Methyl chloride was removed from the quaternary chloride by slowly heating 5 gm. in a distillation flask in a vacuum kept below 1 mm. Excessive heating caused much frothing so the operation was conducted very slowly. There was considerable deep-seated decomposition and an appreciable amount of non-volatile resin remained in the flask. The crude distillate was dissolved in a small volume of methanol and an excess of dilute aqueous nitric acid was added. After removal of the methanol the turbid mixture was filtered through a wet filter and the acid filtrate extracted several times with choloroform to remove non-basic material. The tertiary base was then liberated by the addition of an excess of sodium hydroxide and the base extracted with chloroform. The dried extract was freed of chloroform by repeated evaporation with ethyl alcohol and then poured into a hot dilute solution of picric acid. A small amount of insoluble resin was filtered off and the filtrate slowly cooled. The picrate was thus obtained in pale yellow slender needles melting at 167°C., which after one recrystallization melted alone or admixed with a specimen obtained from the methylation of tryptamine at 168°C.

The purified picrate from 5 gm. of the quaternary chloride was suspended in a small volume of boiling water and treated with an excess of sodium hydroxide. The cooled mixture was extracted with ether and the extract thoroughly washed with aqueous sodium hydroxide and dried over potassium carbonate. Removal of the ether yielded a pale yellow oil (1.6 gm.) which solidified completely in a short time. After pressing out on tile and washing with a mixture of ether and petroleum ether it melted sharply at 47°C. The free base was obtained only in very fine ill-defined needles. It is extremely soluble in all organic solvents with the exception of petroleum ether.

A small amount treated with an excess of methyl iodide in methanol yielded the characteristic micaceous plates of the quaternary iodide, which after one recrystallization from methanol melted at 197°C., alone or admixed with an authentic specimen.

The hydrochloride could be obtained only as a pale yellow resin, which when dried in a vacuum desiccator over potassium hydroxide became porous and brittle. Analysis: Calcd. for $C_{12}H_{17}N_2Cl$; Cl, 15.79%. Found: Cl, 15.44%.

2:2-Dimethyl-3-benzoyl-2:3:4:5-tetrahydro-3-carboline

A solution of 4 gm. of tryptamine in 40 cc. of dry acetone was treated with an excess of methyl iodide. Some heat was evolved but no precipitation occurred. After several hours the acetone was removed on the steam bath, water was added and the slightly turbid solution filtered through a layer of charcoal. The colorless filtrate was basified with excess KOH and the precipitated bases removed by extraction with a mixture of ether and chloroform. A small amount of insoluble crystalline material was obtained which proved to be the quaternary iodide, m.p., 197°C.

The ether-chloroform extract was dried over sodium sulphate, evaporated somewhat and heated for four hours under reflux with an excess of benzoyl chloride and potassium carbonate. The chloroform solution was thoroughly washed with water and with dilute hydrochloric acid and dried over potassium carbonate. On evaporation and treatment with a little acetone and ether, the chloroform solution yielded about 0.7 gm. of very stout brilliant octahedra which after recrystallization from acetone, in which the substance is sparingly soluble, melted sharply at 285°C. Analysis: Calcd. for $C_{20}H_{20}ON_2$; C, 78.94; H, 6.58; N, 9.21%. Found: C, 78.77; H, 6.57; N, 9.25%.

3-(β-Phthalimidoethyl)-1-methylindol

A mixture of 7 gm. of crude 1-methyltryptamine and 12.5 gm. of phthalic acid was slowly heated to 230°C. in an oil bath. The somewhat cooled mixture was treated with 100 cc. of boiling alcohol and the solid broken up with a glass rod. The mixture was then heated under reflux for an hour, cooled, filtered and the solid washed with cold alcohol; yield, 10 gm. A small amount was recrystallized by solution in a large volume of hot acetone and rapidly evaporating the filtrate. On cooling, a solid mass of minute needles was obtained which after filtering, washing with alcohol, and drying melted sharply at 177.5°C. The substance is practically insoluble in cold alcohol and only sparingly in hot acetone. Analysis: Calcd. for $C_{19}H_{16}O_2N_2$; C, 75.00; H, 5.26; N, 9.21%. Found: C, 74.92; H, 5.36; N, 9.35%.

The phthalimido derivative when treated with hydrazine hydrate in the usual manner yielded 1-methyltryptamine quantitatively; b.p., 154°C./1 mm. All attempts to obtain the latter in a crystalline condition failed. Exposure in a sealed tube to the native climatic conditions for six months during which time the temperature frequently fell to -30°C. failed to cause crystallization.

The hydrochloride is readily obtained in colorless needles by neutralizing a solution of the base in alcohol with hydrochloric acid and cautiously adding acetone. Recrystallized from methanol-acetone the salt melts sharply at 198°C.

1-Methyl-2-phenyl-4:5-dihydro-3-carboline

A solution of 2.78 gm. of benzoyl-1-methyltryptamine in 35 cc. of chloroform was heated under reflux with 10 gm. of phosphorus oxychloride for one hour. The solvent and excess oxychloride were largely removed by evaporation in a current of air and the residue decomposed with ice. Some residual chloroform was then boiled off, and the turbid solution filtered through a layer of charcoal. The intensely yellow filtrate was basified and the base extracted with ether, the extract was dried over sodium sulphate, evaporated to a small volume and crystallization induced by cautious addition of petroleum ether. The microscopic crystalline base was filtered off and washed with ether-petroleum ether (1:1) and finally with petroleum ether; yield, 2.3 gm. The substance is colorless and melts sharply at 94°C. Alcohol readily dissolves it to a yellow solution with a pale greenish fluorescence. Analysis: Calcd. for $C_{18}H_{16}N_2$; C, 83.08, H. 6.15, N. 10.77%. Found: C, 83.02; H, 6.14; N, 10.88%.

The hydrochloride readily crystallizes from alcohol-ether or alcohol-acetone

in golden yellow elongated plates, melting at 237°C. to an orange-colored liquid which resolidifies on cooling.

1-Methyl-2-phenyl-3-carboline

A small amount of the dihydro-base was dissolved in dilute sulphuric acid and treated while hot with a solution of chromic acid. A very sparingly soluble chromate was precipitated which gradually dissolved as oxidation proceeded. The filtered solution was basified and the mixture of chromium hydroxide and base filtered off, washed with water, dried and extracted with hot ethyl acetate. The base obtained from the extract did not crystallize. It was converted into the hydrochloride and the latter recrystallized from acetone by adding ether to the concentrated solution. The hydrochloride melts at 278°C. and is very soluble in water or alcohol, yielding a solution with an intense bluish fluorescence. Analysis: Calcd. for $C_{18}H_{15}N_2Cl$; C, 73.34; H, 4.75; Cl, 12.05%. Found: C, 73.02; H, 5.12; Cl, 12.05%.

The picrate is insoluble in water and was recrystallized from hot alcohol in which it is sparingly soluble; m.p., 234°C.

Diethyl β-Cyanopropionacetal

Six moles (275 gm.) of absolute alcohol contained in a flask provided with an inlet tube, a dropping funnel, and a calcium chloride tube, was treated with a stream of dry gaseous hydrogen bromide until 2.75 moles (220 gm.) had been absorbed. The mixture had to be kept cool during this addition since an elevation of temperature converts a portion of the alcohol into the bromide. The mixture was then cooled in salt and ice and 2.5 moles (140 gm.) of acrolein was slowly added, care being taken to prevent local superheating. The mixture was allowed to remain in ice overnight, neutralized with an excess of precipitated calcium carbonate, and about 300 cc. of dry ether was added. The mixture was again allowed to remain in ice overnight and the ethereal layer then decanted from the pasty calcium bromide solution. A small amount of calcium carbonate was added to the ether solution and the solvent then removed *in vacuo*. To the residue, dissolved in 300 cc. of methanol, there was then added 3 gm. of sodium iodide and 2.75 moles (138 gm.) of sodium cyanide dissolved in the minimum volume of water. The mixture was boiled under reflux for 15 to 16 hr., the solvent distilled off on a steam bath and the residue extracted with about 600 cc. of ether in several portions. The extract was freed of ether and the residue fractionally distilled *in vacuo*, through a good glass column. Two main fractions were obtained: (I) up to 97°C./15 mm. (II) 97 to 108°C./15 mm. (mostly at 104-106°C.). Each fraction was redistilled and the higher portion of the first added to the second. The second fraction consisted of the desired nitrile and was conveniently collected over a 2° range. Some boiling points (not corrected) with the corresponding pressures were: 84-85°C./7 mm.; 97°C./11 mm.; 105°C./15 mm. The lower fraction contained a considerable amount of unchanged bromide which may conveniently be added to a subsequent preparation. The yield of nitrile varied from 40 to 60%, depending upon the amount of unchanged bromide recovered.

Diethyl γ-Aminobutyracetal

A solution of 79 gm. of β-cyanopropionacetal (0.5 mole) in 1000 cc. of absolute alcohol was treated with 70 gm. of sodium (3 moles) in large pieces, the mixture being cooled in running water. When the first vigor of the reaction had subsided, the flask was heated on a steam bath until all the sodium had dissolved. The somewhat cooled mixture was then treated with 200 cc. of water and as much alcohol as would distil was removed on a steam bath through a short column. The first distillate was discarded. The residue was distilled under reduced pressure until no more distillate could be obtained, and the distillate fractionated through a good column from a steam bath. The residue was then distilled through a long column *in vacuo*. With considerable care this distillation may be so carried out that a very sharp separation of the water is possible. The yield of a product of 3° boiling range was 67 to 68 gm. (85% of theory).

When methanol was substituded for ethyl alcohol the mean yield of four runs was 71%. The proportions were: nitrile, 157 gm.; methanol, 1500 cc.; sodium, 140 gm. The pure substance boils at 84°C./11 mm. or at 93°C./15 mm.

Tryptamine

The following procedure has been repeatedly followed after numerous trials using slightly different proportions or different conditions, and has been found to give the pure base in consistent yields.

A mixture of 80 gm. (0.5 mole) of γ-aminobutyracetal and 55 gm. of pure phenylhydrazine contained in a 1000-cc. round-bottomed flask is treated with 68 gm. of finely ground anhydrous zinc chloride. There is a moderate exothermal reaction and the mixture turns pale brown. An upright condenser is attached but no water is run into it. The flask is then gently heated by rotating it over a free flame and the greater portion of the alcohol formed in the reaction is distilled through the condenser. Further cautious heating is continued, preferably with a little local heating occasionally, until a rather vigorous exothermal reaction ensues. Water is then rapidly run into the condenser and the flask removed from the source of heat. When the reaction has subsided the fluid dark-brown mass is run onto the sides of the flask in order to present as great a surface as possible in the subsequent solution of the material. When sufficiently cooled, 60 cc. of acetic acid and 100 cc. of water is added and the mixture gently heated over a free flame until solution is complete. Water (about 600 cc.) is then added (the addition of the water precipitates a dark resinous material which need not be filtered off at this stage) and the zinc is precipitated with a stream of hydrogen sulphide. The zinc sulphide and resin are filtered off through a layer of charcoal and the pale yellow filtrate added to a concentrated solution of 100 gm. of sodium hydroxide. The oil which separates crystallizes on cooling or with great facility on seeding with a crystal of tryptamine. After remaining in the ice chest overnight the amine is filtered off, washed with cold water, and dried in a vacuum desiccator over potassium hydroxide. The yield of this product is 55 gm. (this and the subsequent yields are the average of four runs, the variation being less than

5%). The crude product is distilled in a vacuum, preferably of 2 mm. or less, from an oil bath and is thus obtained as a pale yellow viscous liquid which rapidly solidifies; yield, 47.4 gm. Further purification is conveniently effected by solution in the minimum volume of hot chloroform and slow cooling. There is thus obtained an almost colorless product consisting of stout polyhedra melting at 118°C.; yield, 41 gm. (51% of theory). The mother liquor on removal of the solvent and distillation of the residue yields a further small amount of equally pure product.

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